Original Article

Histomorphological Evaluation of Desmoplastic Tumor Stroma in Malignant Ovarian Surface Epithelial Tumors

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Abstract

Background: Ovarian cancer is the 8th most common cancer in women worldwide. Tumor budding is defined as a type of invasive growth in carcinomas with either a single tumor cell or a cluster of up to four cells at the invasive tumor front and is associated with epithelial–mesenchymal transition. A reactive stroma rich in cancer-associated fibroblasts is associated with higher tumor grade and poorer prognosis in breast, colorectal, and oral cancers. **Aims and Objectives:** The present study was conducted to highlight the prognostic significance of tumor budding and fibrotic cancer stroma in malignant ovarian surface epithelial tumors with known prognostic parameters. **Materials and Methods:** This was a retrospective cross-sectional study conducted over a 2-year period, in which all histologically diagnosed cases of malignant ovarian surface epithelial tumors who underwent surgery were included. The fibrotic stroma was classified into three distinct categories – mature, intermediate, and immature. The number of tumor buds was counted at the invasive front of the tumor and graded based on the number of buds – 0–5, 5–9, and ≥10 buds. **Results:** Among the 50 cases, 32% (16 cases) had mature stroma, whereas 30% (15 cases) and 38% (19 cases) had intermediate and immature stroma, respectively. Although a significant association could not be established between tumor budding and stroma grade, a fair agreement was established between them. A significant association could be established between histological grade with both tumor budding (P = 0.03) and fibrotic stroma grade (P = 0.02). **Conclusion:** The study highlighted the role of stromal response in malignant surface epithelial tumors of the ovary since a higher-grade tumor was associated with an immature stroma, whereas a lower-grade tumor was associated with a mature stroma.

Keywords: *Desmoplastic stroma, malignant ovarian surface epithelial tumors, tumor budding*

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Introduction

Ovarian cancer is the 8th most common cancer in women worldwide accounting for almost 313,959 new cases and 20,7252 deaths in 2020. According to Globocan 2020, it is the 3rd most prevalent cancer among females with 45,701 new cases and 32,077 deaths in India with a death rate of 3.8%.[1]

The WHO classification of ovarian tumors includes a wide spectrum from surface epithelial, germ cell tumors, sex cord-stromal tumors, and metastatic lesions.[2] The risk factors associated with ovarian surface epithelial tumors include family history, BRCA1/2 mutations, Lynch syndrome, older age, number of ovulatory cycles, nulliparity, endometriosis, central obesity, and smoking. Reduced ovulation, pregnancy, lactation, and tubal ligation decrease the risk of ovarian cancer.[3]

Tumor budding is defined as a type of invasive growth in carcinomas with either a single tumor cell or a cluster

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of up to four cells at the invasive tumor front. The buds are a part of the tumor microenvironment and are associated with epithelial-mesenchymal transition.[4]

The prognostic value of tumor budding is evident from the inclusion of this feature in the WHO classification 2019 and as a recommended element in the College of American Pathologists on reporting protocols for colorectal cancer.[5] The association of higher tumor budding with various prognostic parameters such as higher T-stage, lymph node metastasis, reduced overall survival, and risk of recurrence has been evaluated in head and neck, lung, breast, endometrial, gastroesophageal, pancreaticobiliary, and colorectal cancer.[6,7]

In the recent years, cancer outlook has changed significantly that the tumor is not only a group of malignant cells but also a complex tumor microenvironment. The stromal component of this microenvironment is composed of cancer associated fibroblasts (CAF), macrophages, neutrophils, NK cells, regulatory T-cells, platelets, and mast cells which interact with each other as well as the tumor cells through various cytokines, chemokines, growth factors – epidermal growth factor, vascular endothelial growth factor, IL-6, and IL-17.[8] CAFs are important in the tumor initiation and progression through remodeling of extracellular matrix and modulation of tumor microenvironment by autocrine and paracrine signaling. A reactive stroma rich in CAFs is associated with higher tumor grade and poorer prognosis in breast, colorectal, and oral cancers.[8,9]

Morphologically, desmoplastic stroma is categorized into mature, intermediate, and immature based on the presence of extracellular matrix components such as keloid-like collagen and myxoid stroma.[10]

The present study was conducted to highlight the prognostic significance of tumor budding and fibrotic cancer stroma in malignant ovarian surface epithelial tumors with known prognostic parameters.

Materials and Methods

This was a retrospective cross-sectional study conducted over a 2-year period, in which all histologically diagnosed cases of malignant ovarian surface epithelial tumors who underwent surgery were included in the study.

Exclusion criteria

Patients who received any neoadjuvant therapy were excluded from the study.

The resected specimens of malignant ovarian surface epithelial tumors received in the histopathology laboratory over a 2-year period were taken and clinical details (age, gender, and site) were retrieved from the requisition form. Fifty cases which fulfilled the inclusion criteria were taken up for the study. Routine histopathological processing was done, followed by hematoxylin and eosin staining and immunohistochemistry for cytokeratin (for tumor budding) and smooth muscle actin (for evaluation of myofibroblasts).

Evaluation of tumor budding and fibrotic stroma

The entire length of the invasive front of tumor in H- and E-stained slides was scanned at low power to look for the presence/absence of tumor buds. Hotspot areas with maximum budding activity were identified, and the number of buds was counted at higher magnification (×20 or ×40).[6] The number of tumor buds was scored as follows:

a. 0–4 tumor buds – 1 (low grade)
b. 5–9 tumor buds – 2 (intermediate grade)
c. 10 or more tumor buds – 3 (high grade).

The fibrotic stroma was classified as follows:

a. Mature – when it comprised fine elongated collagen fibers with fibroblasts stratified into multiple layers without keloid-like collagen or myxoid stroma (Grade 1)
b. Intermediate – when broad bands of collagen with bright eosinophilic hyalinization were intermingled with mature collagen fibers (Grade 2)
c. Immature – when keloid-like collagen was randomly distributed in a myxoid stroma (Grade 3).[10]

Statistical analysis

The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, version 25.0. Fisher's exact test was done for the comparison of the variables along with interrater kappa agreement to assess the strength of agreement between tumor budding and grade of fibrotic stroma. $P < 0.05$ was considered to be statistically significant.

Results

Among the fifty cases of malignant ovarian surface epithelial tumors, the mean age of presentation was 57.5 years (range: 38–71 years). Based on the histological diagnosis, there were 66% of serous adenocarcinomas (33 cases), 20% mucinous (10 cases), and 14% endometrioid (7 cases). The majority of lesions were high grade (24 cases – 48%), followed by low (18 cases – 36%) and intermediate grade (8 cases – 16%) [Figure 1].

According to the FIGO staging, there were 20% (10 cases), 50% (25 cases), and 30% (15 cases)

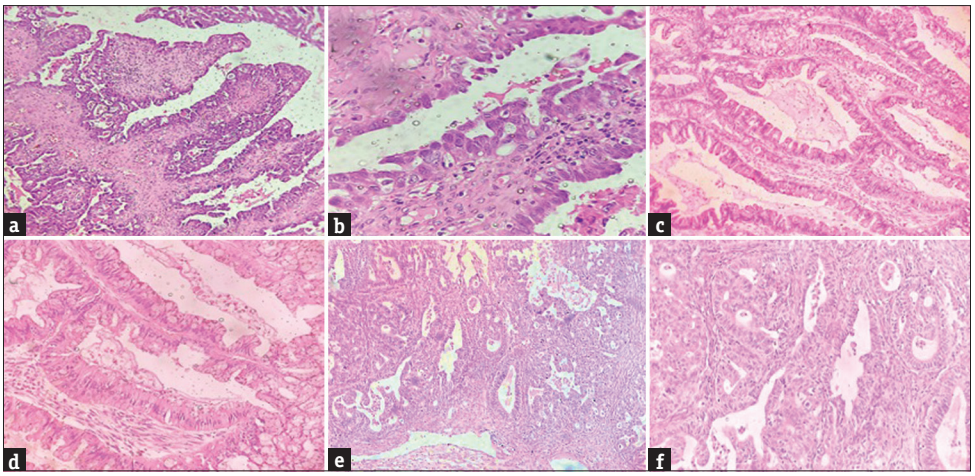

Figure 1: (a and b) Hematoxylin- and eosin-stained sections of serous papillary adenocarcinoma (×100, ×200), (c and d) Hematoxylin- and eosin-stained sections of mucinous adenocarcinoma (×100, ×200), (e and f) Hematoxylin- and eosin-stained sections of endometrioid adenocarcinoma (×100, ×200)

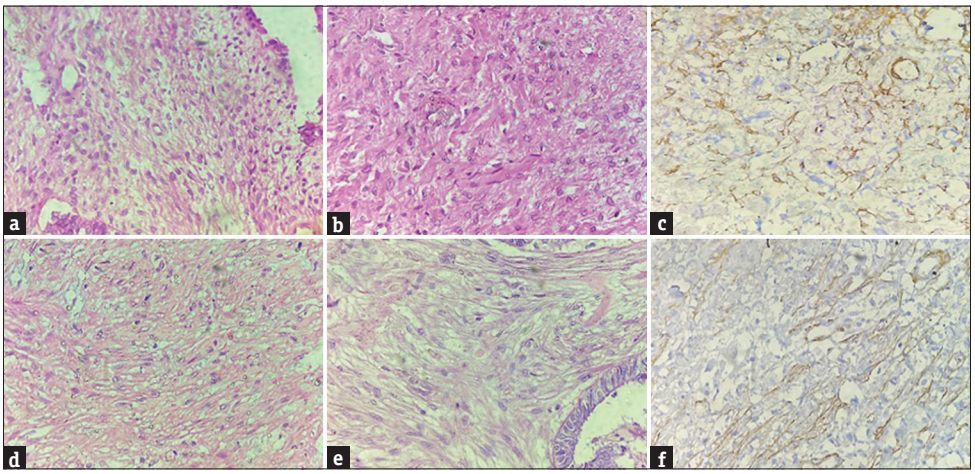

Figure 2: (a) Hematoxylin- and eosin-stained sections of mature stroma with parallel arranged collagen bundles (×200), (b) Intermediate stroma with broad keloid-like bundles of eosinophilic collagen intermingled with mature collagen fibers on hematoxylin and eosin, and (c) SMA immunohistochemistry (×200), (d and e) Immature stroma with randomly oriented keloid-like bundles surrounded by myxoid stroma on hematoxylin and eosin, and (f) SMA immunohistochemistry (×200). SMA: Smooth muscle actin

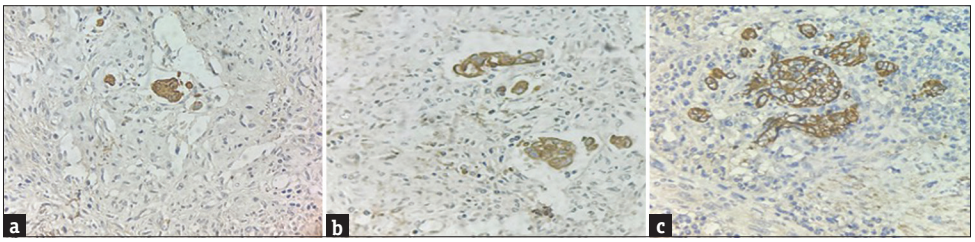

Figure 3: Tumor budding (×400), (a) Grade 1, (b) Grade 2, (c) Grade 3

in Stages I, II, and III, respectively. None of the cases belonged to Stage IV.

Among the 50 cases, 32% (16 cases) had mature stroma, whereas 30% (15 cases) and 38% (19 cases) had intermediate and immature stroma, respectively [Figure 2]. The number of tumor buds [Figure 3] was maximum with the immature stroma and reduced with the maturity of the stroma [Table 1].

Although a significant association could not be established between tumor budding and stroma grade, fair agreement was established between them. Among the 16 cases diagnosed with Grade I stroma, 9 cases had similar grade in tumor budding. Among the 19 cases diagnosed with Grade III stroma, 12 had similar findings in tumor budding. The overall concordance rate and discordance rate between tumor budding and stroma were 54% and 46%, respectively.

Table 1: Inter-rater kappa agreement between tumor budding and fibrotic stroma

Tumor budding	Stroma grade			Total, *n* (%)	*P*	Kappa
	Grade 1 (*n*=16), *n* (%)	Grade 2 (*n*=15), *n* (%)	Grade 3 (*n*=19)			
Grade 1	9 (18)	4 (8)	4 (8)	17 (34)	0.07	0.303
Grade 2	3 (6)	6 (12)	3 (6)	12 (24)		
Grade 3	4 (8)	5 (10)	12 (24)	21 (42)		
Total	16 (32)	15 (30)	19 (38)	50 (100)		

A significant association could be established between histological grade with both tumor budding (P = 0.03) and fibrotic stroma grade (P = 0.02).

However, no such association was observed between stage of tumor with budding (P = 0.39) and stroma grade (P = 0.37).

Discussion

The cross-talk between the tumor cells and neoplastic stromal cells is involved in the acquired ability for invasive growth and metastasis through epithelial–mesenchymal transition.[11] The process of dissociation of tumor cells, followed by dedifferentiation is the initial step in tumor invasion and metastasis by interaction with stroma at the invasive edge.[12]

Ovarian cancer is the third most common cancer among women in India. The majority of the women in our study belonged to the age group of 51–60 years of age with a mean age of 57.5 years (range of 38–71 years). Chen *et al*. evaluated tumor stroma ratio in ovarian surface epithelial tumors and reported a median age of 55 years with a range of 21–79 years.[13]

In the present study, serous adenocarcinoma (66%) was the most common diagnosis, followed by mucinous (20%) and endometrioid adenocarcinoma (14%) which was similar to the distribution of other studies.[13] Based on differentiation, there was an almost equal distribution of low- and intermediate-grade (52%) and high-grade (48%) ovarian carcinomas which was concordant to the previous data.[13]

Chen *et al*. reported 71% Stage III-IV and 29% Stage I-II tumors, respectively.[13] However, the present data reported an opposite distribution with 70% Stage I-II and 30% Stage II-IV cancers.

Tumor budding was first described in the early 1950s as the sprouting at the leading of the tumor.[14] Tumor budding has been evaluated in colorectal, lung adenocarcinoma, anal, esophageal, and head-and-neck squamous cell carcinoma. Tumor budding is of two types – peritumoral (evaluated at the leading edge of the tumor) and intratumoral (budding within the tumor). It is known to be an independent prognostic marker and is associated with poor prognosis, lymph nodal metastasis, and locoregional recurrence.[15,16]

No significant data are available on the evaluation of tumor budding in ovarian surface epithelial tumors. The present study is the first to evaluate tumor budding with fibrotic stroma and clinicopathological parameters in ovarian cancers. Grade 3 tumor budding (42%) was observed in the majority of the ovarian cancers, followed by Grade 1 (34%) and Grade 2 (24%), respectively.

There have been few studies on colorectal cancer which suggest the correlation between the intensity of tumor budding and maturation of the fibrotic stroma.[17-19] The desmoplastic stroma is categorized into mature, intermediate, and immature based on the presence of keloid-like collagen and myxoid stroma at the invasive edge of the tumor. The intermediate and immature stroma is mostly confined to the advancing edge of the tumor and seems to be transitory phenotypes which allow dedifferentiation of cancer cells. The immature stroma is also known to inhibit the immune cells from reaching the tumor area as the myofibroblasts create a physical barrier around the tumor, thus leading to a poorer prognosis.[10] However, mature stroma is a more stable phenotype and may restrict the invasion by tumor cells.[20,21]

In the present study, most of the cases demonstrated an immature Grade 3 type of stroma (38%), followed by Grade 1 (32%) and Grade 2 (30%). Although a significant association (P = 0.07) was not observed between budding and stroma grade possibly due to the small sample size, a fair interrater kappa agreement was noted. However, the grade of the tumor showed a positive association with both tumor budding (P = 0.03) and stroma grade (P = 0.02).

Kaur *et al*. demonstrated a significant association between tumor budding and stroma grade in colorectal cancer.[22] Chen *et al*. have previously evaluated the tumor stroma ratio in ovarian tumors and observed that stroma-rich tumors are associated with advanced stage, lymph nodal metastasis, and recurrence.[13]

However, there has been no study on the evaluation of stroma type and tumor budding in ovarian cancer.

Conclusion

The present study concluded that, in malignant ovarian surface epithelial tumors, a high-grade tumor was associated with an immature stroma, whereas a low-grade tumor was associated with a mature stroma. A fair agreement was established between tumor

budding and stroma grade. The tumor stroma plays an important role in tumor progression and metastasis. Therefore, more studies with greater sample size are required to understand the role of extracellular matrix and tumor stroma in ovarian cancer to help identify prognostic markers.

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Conflicts of interest

There are no conflicts of interest.

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