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ORIGINAL PAPER

Peritumoral Retraction Clefting Correlates with Advanced Stage Squamous Cell Carcinoma of the Esophagus

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Abstract The present study was designated to analyze correlation between the presence and extent of peritumoral retraction clefting and various clinicopathologic features in esophageal squamous cell carcinoma (ESCC), and to possibly establish the significance of this phenomenon in ESCC. Fifty-four consecutive patients with advanced ESCC were included in the study. The presence of peritumoral retraction clefting was classified on the basis of the proportion of tumor nests exhibiting this phenomenon. Tumors with clefts that affected up to 25% of tumor nests were classified as group I; with clefts that affected >25% to 50% of tumor nests as group II; with clefts that affected >50% to 75% of tumor nests as group III; and tumors with clefts that affected more than 75% of tumor nests were classified as group IV. Statistical analysis showed a correlation between presence and extent of peritumoral clefting and lymph node metastasis. T3 tumors and tumors with lymph node metastasis had significantly more pronounced peritumoral clefting compared with T2 tumors and tumors without lymph node metastasis. The presence of peritumoral clefting was not associated with the number of affected lymph nodes. There was no correlation between the presence and extent of peritumoral clefting with patient age and sex, and tumor location, diameter and grade. The association of peritumoral retraction clefting in ESCC with local invasiveness and lymph node metastasis indicated that peritumoral clefting could be a simple and useful morphological feature of tumor aggressiveness and may contribute to the pathological and clinical assessment of patients with ESCC.

Keywords Esophagus · Metastases · Peritumoral clefting · Squamous cell carcinoma · Tumor stage

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Introduction

Esophageal squamous cell carcinoma (ESCC) is an aggressive tumor with a high mortality rate and an increasing incidence in Central and Eastern Europe [1]. The incidence and mortality of ESCC in Croatia in the year 2005 was 4.8 and 5.2 per 100,000 persons, respectively [2]. One of the most important clinicopathologic features that affect the survival of patients with ESCC is infiltration of local tissue and metastasis to regional lymph nodes and distant organs peritumoral clefting [3]. In most solid tumors including ESCC, the spread of cancer cells *via* the lymphatics to regional lymph nodes is an important early event during tumor progression [4]. The exact mechanisms of tumor cell spread to the lymphatic system remain unresolved, and it is not clear whether lymphatic spread is an active or passive process, and whether it depends on tumor-induced lymphangiogenesis or invasion of pre-existing lymphatic vessels [5]. It is also well known that tumor–stroma interaction is important in regulating local cancer invasion

and metastasis, and may have prognostic and diagnostic implications [6, 7].

Peritumoral retraction artifact or clefts separating tumor cells or nests from the adjacent stroma can frequently be seen on histologic sections of different tumors [8–13]. Peritumoral retraction clefting appears in tissue sections as an empty space partially or completely surrounding nests of tumor cells and can pose difficulty in distinguishing such foci from lymphovascular invasion. The occurrence of peritumoral retraction clefting in tissue sections was mostly considered to be a consequence of inadequate fixation, tissue processing or cutting, and diagnostic pathologists have paid little attention to this phenomenon. Some recent studies have shown that retraction clefting could have diagnostic and prognostic significance [8–13]. Kruslin et al. [8, 12] and Ulamec et al. [13] showed that retraction clefting could be helpful in the diagnosis of prostatic carcinoma. The presence of clear spaces around tumor nests has also been suggested as a diagnostic criterion of invasive peritoneal implants of ovarian serous borderline tumors [9]. Recent studies by Acs et al. [10] and Irie et al. [11] pointed to the prognostic and diagnostic significance of peritumoral retraction clefting in breast carcinoma.

We have also observed that some ESCC show prominent retraction clefting on routinely prepared hematoxylin and eosin stained sections. The present study was designated to analyze correlation between the extent of peritumoral retraction clefting and various clinicopathologic features in ESCC, and to possibly establish the significance of this phenomenon in ESCC.

Materials and Methods

Fifty-four consecutive patients with advanced ESCC (associated with invasion to the muscularis propria layer or adventitia of the esophagus) who underwent radical surgery were included in the study. None of the patients received preoperative chemotherapy or radiotherapy. There were eight (14.8%) women and 46 (85.2%) men, aged 38 to 73 years (mean 57.2 years). Tumor diameter varied from 1.4 to 10 cm (mean 3.6 cm). Eighteen (33.3%) tumors were located in upper, 31 (57.4%) in middle and five (9.3%) in lower part of the esophagus. The pathologic stage of each cancer at the time of operation was defined according to the TNM system [14], and each lesion was graded histologically based on the parameters of mitotic activity, anisonucleosis and degree of differentiation according to the World Health Organization classification [3]. TN stage and tumor grade in 54 study patients are shown in Table 1. All lymph nodes dissected were completely processed for pathological examination. The number of dissected lymph nodes varied from three to 24 (mean 10.5 nodes). Three to 22 lymph nodes (mean 9.8 nodes) were dissected in cases without lymph node metastasis, and four to 24 lymph nodes (mean 11.1 nodes) in cases with lymph node metastasis. In cases with lymph node metastasis, one to 12 nodes (mean 3.2 nodes) were affected by tumor.

Table 1 TN stage and tumor grade in 54 patients with squamous cell carcinoma of the esophagus

	Number of cases
T stage	
T2	24 (44.4%)
T3	30 (55.6%)
N stage	
N0	25 (46.3%)
N1	29 (53.7%)
Tumor grade	
G1	8 (14.8%)
G2	29 (53.7%)
G3	17 (31.5%)

Gross specimens were fixed overnight in 10% buffered formalin. Tumors were sampled in three to seven sections, which were 3 to 4 mm thick and additionally fixed in 10% buffered formalin approximately 24 h. After fixation specimens were embedded in paraffin, cut at 5 μm thickness, and routinely stained with hematoxylin and eosin.

In each case, the available routinely prepared hematoxylin and eosin stained sections were reviewed, the diagnosis was confirmed and slides with the deepest portion of tumor penetration were selected and included in the study. Tumor cells and nests surrounded by a clear space without an endothelial lining separating tumor cells from the adjacent stroma were considered as retraction clefting. The presence of peritumoral retraction clefting was classified on the basis of the proportion of tumor nests exhibiting this phenomenon. Tumors with clefts that affected up to 25% of tumor nests were classified as group I; with clefts that affected >25% to 50% of tumor nests as group II; with clefts that affected >50% to 75% of tumor nests as group III; and tumors with clefts that affected more than 75% of tumor nests were classified as group IV [11]. All samples were examined independently by three observers (T. B, I. P. and D. T.), and any difference was resolved by a joint review.

The extent of peritumoral retraction clefting was compared with the patient's sex and age, and tumor diameter, location, grade, depth of invasion and presence of lymph node metastasis. Mann Whitney *U*-test was used for between-group comparison, the χ^2-test was used to estimate the degree of association between the selected variables and Fisher's exact test was used for determination of significant differences between analyzed groups. Level of significance was set at $p<0.05$.

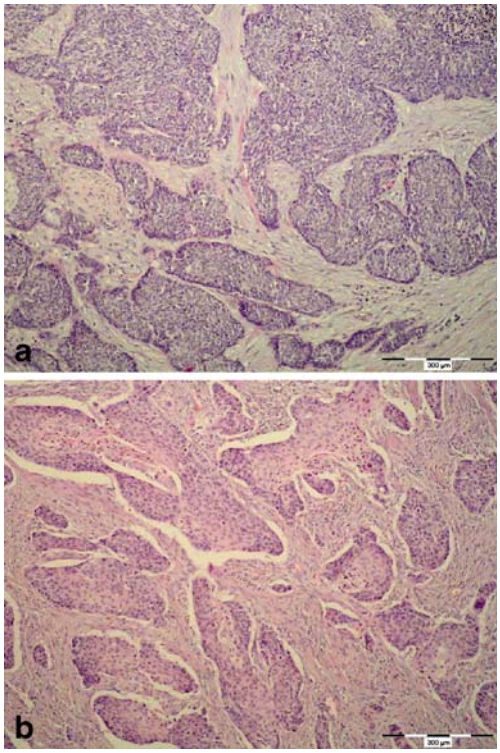

Fig. 1 Esophageal squamous cell carcinoma with sparse (**a**) and prominent (**b**) peritumoral retraction clefting (H&E, 100×)

Results

Peritumoral clefting was observed in all cases examined. Out of 54 patients, 20 (37.0%) were in group I (Fig. 1a), 17 (31.5%) in group II, 14 (25.9%) in group III, and three (5.6%) in group IV (Fig. 1b). There was no correlation between the presence of peritumoral clefting and patient age and sex, tumor location and diameter ($p>0.05$). The correlation between the presence of peritumoral clefting and TN stage and tumor grade is shown in Table 2. Out of 24 T2 stage tumors, 14 (58.3%) showed peritumoral clefting in up to 25% of tumor nests and only three (12.5%) in more than 50% of tumor nests. Peritumoral clefting that affected more than 50% of tumor nests was recorded in 46.7% of T3 stage tumors, in most of them (40.0%) affecting between 50% and 75% of tumor nests. Statistical analysis showed a correlation between the presence of peritumoral clefting and T stage of tumor ($p<0.05$). The number of dissected lymph nodes was not statistically significantly different between patients with (N1) and patients without (N0) lymph node metastasis ($p>0.05$). Peritumoral clefting affecting more than 50% of tumor nests was recorded in only two (8.0%) N0 tumors and 15 (51.7%) N1 tumors. None of N0 tumors and three (10.3%) N1 tumors showed peritumoral clefting affecting more than 75% of tumor nests. Statistical analysis showed a correlation between presence and extent of peritumoral clefting and lymph node metastasis ($p<0.05$). In addition, T3 tumors and N1 tumors had a statistically significantly more pronounced peritumoral clefting compared to T2 tumors and N0 tumors ($p<0.05$). The presence of peritumoral clefting did not correlate with the number of affected lymph nodes ($p>0.05$). The distribution of peritumoral clefting was similar in well (G1), moderately (G2) and poorly (G3) differentiated tumors. Statistical analysis showed no significant differences between the groups analyzed ($p>0.05$).

Discussion

Peritumoral retraction artifacts present in sections of formalin fixed, paraffin-embedded tissue samples are a well known phenomenon, especially in basal cell carcinoma [15]. Similar changes, also called peritumoral retraction clefting or peritumoral halos, were first described by Halpert et al. [16, 17] in prostatic carcinoma. For quite a long time, these findings received little attention and were mostly considered as a technical artifact during laboratory procedure. Recently, some authors have pointed to the diagnostic and prognostic significance of this phenomenon in prostate carcinoma, urinary bladder carcinoma, ovarian serous tumors and breast

Table 2 Correlation between the presence of peritumoral clefting and TN stage and tumor grade in 54 patients with squamous cell carcinoma of the esophagus

Peritumoral clefting	TN stage				Tumor grade		
	T2	T3	N0	N1	G1	G2	G3
Group I	14 (25.9%)	6 (11.1%)	17 (31.5%)	3 (5.6%)	6 (11.1%)	10 (18.5%)	4 (7.5%)
Group II	7 (12.9%)	10 (18.5%)	6 (11.1%)	11 (20.3%)	1 (1.9%)	11 (20.3%)	5 (9.2%)
Group III	2 (3.7%)	12 (22.3%)	2 (3.7%)	12 (22.2%)	0 (0%)	7 (12.9%)	7 (12.9%)
Group IV	1 (1.9%)	2 (3.7%)	0 (0%)	3 (5.6%)	1 (1.9%)	1 (1.9%)	1 (1.9%)
Total	24 (44.4%)	30 (55.6%)	25 (46.3%)	29 (53.7%)	8 (14.9%)	29 (53.6%)	17 (31.5%)

Group I tumors with clefts affecting up to 25% of tumor nests, *Group II* tumors with clefts affecting >25% to 50% of tumor nests, *Group III* tumors with clefts affecting >50% to 75% of tumor nests, *Group IV* tumors with clefts affecting more than 75% of tumor nests

carcinoma [8–13, 18]. Acs et al. [10] correlated the appearance of peritumoral clefting and clinicopathological characteristics in breast carcinoma and concluded that extensive retraction artifact correlated with lymphatic invasion and nodal metastasis and predicted poor outcome in early stage breast carcinoma. Irie et al. [11] showed that peritumoral artifacts were more pronounced in invasive compared to in situ breast carcinoma. Similar findings were observed by Kruslin et al. [19] in the prostate where peritumoral clefting was more prominent around malignant glands compared to glands with prostatic intraepithelial neoplasia. The origin and the biological mechanisms of this phenomenon as well as its biological or clinical significance remain to be clarified. Kruslin et al. [8, 12, 19] considered peritumoral clefting in prostate cancer as a sign of altered stroma–tumor interaction or degradation of basement membrane in malignant glands. Tomas and Kruslin [6] and Tomas et al. [20] pointed to the connection of myofibroblastic stromal changes and expression of laminin and tenascin-C in prostate carcinoma with the appearance of peritumoral artifact. Acs et al. [10] and Irie et al. [11] discussed the possibility that this phenomenon might represent true prelymphatic space involvement by malignant glands rather than the result of tissue fixation or processing.

Several findings support the hypothesis that peritumoral clefting does not represent a simple technical artifact First, in the study of prostate carcinoma and breast carcinoma there was no significant difference in the presence and extent of peritumoral clefting between core biopsy and corresponding excision specimens [10, 12]. Second, peritumoral clefting was much more commonly seen in association with tumor glands than with benign glands present on the same section, even in the same high power field, as demonstrated by Ulamec et al. [13].

Several morphological and molecular markers have been proposed as predictive and prognostic factors in patients with ESCC [21–25]. While molecular methods demand sophisticated and expensive equipment, some simple and inexpensive morphological features show connection with prognosis in patients with ESCC. Ishibashi et al. [21] showed a significant correlation of tumor associated tissue eosinophilia with the presence of vascular invasion, lymph node metastasis and recurrence. Tumor-associated macrophages and tumor-infiltrating lymphocytes also correlated with survival of patients with ESCC [22]. Nakanishi et al. [23] studied tumor nest configuration in ESCC and divided tumor nests into two categories: type A characterized by oval tumor nests with a round margin and type B characterized by asteroid-shaped tumor nests with a spiculated margin. Tumors with predominantly type B nests occurred more frequently in males than in females and were characterized by deeper tumor penetration, lymphatic permeation and lymph node metastasis [23].

In our study, peritumoral retraction clefting correlated with local tumor penetration and lymph node metastasis. Tumors with prominent clefting were mostly in T3 stage and with lymph node metastasis. The number of affected lymph nodes, tumor grade, location and diameter as well as the patient's age and sex showed no correlation with the presence of peritumoral clefting.

It is well known that tumor–stroma interaction plays a significant role in tumor development and progression [26]. Alteration in the extracellular matrix composition and changes in protease activity are among essential factors in tumor growth promotion and invasion [27]. On the contrary, tumor cells can also regulate the development of tumor stroma through expression of growth factors or induction of growth factor receptors in the stroma [28]. We think that peritumoral clefting probably occurs as a consequence of tumor–stroma interaction. Significant correlation was demonstrated between the presence of peritumoral clefting and clinicopathological features associated with an aggressive phenotype in ESCC. Similar results have been reported in breast carcinoma [10].

In conclusion, this study is the first report of the association of peritumoral retraction clefting in ESCC with clinicopathological characteristics that indicate more aggressive behavior such as local invasiveness and lymph node metastasis. Thus, peritumoral clefting could be a simple and useful morphological feature of tumor aggressiveness that may contribute to the pathological and clinical assessment of patients with ESCC.

Further studies in larger patient groups are needed to confirm the reliability of the criterion proposed.

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