Elastosis in Breast Cancer as a Surrogate Marker for Estrogen Receptor Positivity

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Abstract

Objectives: This study aims to describe the occurrence and prognostic value of stromal elastosis in breast carcinoma among Omani female patients using semi-quantitative methods. In addition, evaluation of the diagnostic accuracy of H&E stain method in quantifying elastosis compared to EVG as gold standard was pursued.

Methods: The content of elastic tissue in primary infiltrating carcinomas of breast was assessed using semi-quantitative methods (H&E and EVG stains) in 80 female Omani patients by two independent pathologists. Data of primary breast cancer patients, who were not treated with neoadjuvant therapy from 2009 to 2019 at the Armed Forces Hospital (AFH) of Oman, were collected from medical records. Demographic and clinical data including age, menstrual status, tumor type and grade, ER, PR, HER2-neu status, and Ki-67 index were obtained. Follow-up data including clinical remission, evidence of metastasis, death or lost follow-up were traced from medical records. Statistical analysis was done using SPSS program. Ethical approval was obtained from the Histopathology Department of the AFH.

Results: Among 80 cases studied, 80.0% were diagnosed with invasive ductal carcinoma, not otherwise specified; while 12.6% of the cases were diagnosed with infiltrating lobular carcinoma. Interobserver agreement of grading elastosis on H&E and EVG was strong (Kappa coefficient = 0.858). Using EVG, absent elastosis, grade
1, grade 2, grade 3 was observed in 12.5%, 37.5%, 30%, and 20% respectively. A statistically significant relation between high elastosis and estrogen receptor positivity (\(p = 0.015\)) and negative HER2/neu receptor (\(p = 0.045\)) was observed. No statistically significant relation between elastosis and other entities including menopausal status, tumor type and grade, PR, Ki67 and prognosis. Sensitivity and specificity of quantifying elastosis on H&E stained sections as compared to EVG stain (the gold standard) were 68.75% and 96.88% respectively.

**Conclusions:** Elastosis occurrence varies in different breast cancer populations. Elastosis can be considered as a surrogate marker for estrogen positivity and HER2/neu negativity in breast cancer patients. In addition, H&E stain is considered as an accurate method for quantifying elastosis compared to RVG staining method.

**Keywords:** elastin; breast, neoplasm; survival, receptor estrogen; receptor progesterone; HER2-neu; Ki-67 Antigen; Hematoxylin; Eosin Bluish

**Introduction**

Breast cancer is the most common cancer in women worldwide and a major leading cause of death.\(^1\) However, due to mammogram screening, many cases with asymptomatic lesions are detected early and ultimately the prognosis is much better.\(^2\) Nowadays, there are innumerable studies that are looking for more histological and molecular features in the tumors that may help in reducing the overtreatment and predict better prognosis.\(^2\)

Recently, studies are focusing on the stroma surrounding the malignant cells in breast cancers which provides the support and better growth environment for the tumor cells. The striking component is the accumulation of large amount of elastin fibers in and around the tumor, a status known as elastosis.\(^2\) Elastic fibers are usually seen in H&E stained sections; however, quantifying elastosis requires Elastin Van Gieson stain (EVG) as a gold standard.\(^3\) The amount of these elastic fibers differs according to the nature of the lesion; it increases in benign breast diseases with the increment in the number of hyperplastic cells, and the amount is much more in the malignant lesions.\(^4\)

Due to this relation between elastosis and breast cancer, some authors consider the presence of large aggregates of elastin in breast tissue indicates the presence of cancer.\(^5\) Shivas, Douglas et al were the first to study the elastosis in breast cancer decades ago, and they found
that large amount of elastin in breast lesions is associated with better prognosis and improved survival. Studies have also found an association between elastosis, estrogen receptor (ER) and progesterone receptor (PR), and better response to hormonal therapy.\textsuperscript{2,6} Subsequently, limited studies have addressed the prognostic value of elastosis in primary breast cancer.

For example, Chen Y et al (2014)\textsuperscript{2} found that stromal elastosis is associated with good prognostic markers including ER positivity, HER2 negativity, and lower Ki67.\textsuperscript{2} In addition, they found that high amount of elastosis are seen in morphologically highly differentiated tumors (low histological grade) compared to poorly differentiated tumors (high histological grade).\textsuperscript{2,3} Gupta D and others (2015)\textsuperscript{4} studied the relation between elastosis and the two steroid receptors i.e. ER & PR, and observed large amount of elastin associated with intense estrogen positivity, however, this relation is noticed in a lesser degree with the PR.\textsuperscript{4,6} On the other hand, other studies found that the amount and the extent of elastosis in the stroma within the tumor shown to have little value in recurrence-free survival or prognosis.\textsuperscript{2}

Up to date, no local data is available describing the trend of elastosis among Omani patients. In addition, as stated above, limited literature addressed the prognostic value of elastosis in breast cancer patient, which is still controversial and need further exploration. Furthermore, there is no literature addressing the diagnostic accuracy of H&E stain method in quantifying elastosis.

This study aims to describe the occurrence of elastosis in invasive breast carcinoma among Omani female patients using semi-quantitative methods (Hematoxylin and eosin (H&E) stain and EVG stain. In addition, further investigation of the relationship of elastosis with the prognosis and prognostic markers including ER positivity, PR, HER2/neu receptor, tumor grade, and Ki-67 index was sought. Furthermore, the diagnostic accuracy of H&E stain method in quantifying elastosis compared to EVG was evaluated.

**Material & Methods**

In this retrospective study, data of female patients diagnosed with breast cancer who underwent biopsy or resection surgery in the AFH in Muscat, Oman, between 2009 and 2019 were retrieved from the electronic hospital records. Patients with post neoadjuvant chemotherapy resection were excluded from the study.
Data retrieved from the medical records included age, menopausal status, tumor type and grade, ER, PR, Ki67 and Her2 nue status. Follow-up data including clinical remission, evidence of metastasis, death or lost follow-up was also obtained. The age was further subcategorized into < 30, 30-40 years, 40-50 years, 50-60 years and > 60 years. Tumors were classified as ductal, lobular and others. Tumor grading was done according to the Nottingham criteria. Ki67 was also categorized as ≤ 20% and equal or more than 20%.

H&E stained slides of all patients were retrieved from the pathology department in the AFH. After initial scanning, the faded slides were re-cut and stained again. An additional special stain, which is Elastin Van gieson (EVG) to detect elastosis was done for all cases and considered the gold standard. Both slides (H&E and the EVG stained slides) were examined and evaluated under microscope (the standard procedure) for the amount of elastosis. Figure 1 shows grade 2 elastosis in H&E and the EVG stained slides.

Figure 1: Grade 2 elastosis in H&E stained slide (x40) (A), and the same in EVG stained slide (x20) (B)

Elastosis was graded according to a previously published grading system as illustrated in Table 1. In this study, elastosis was graded using a semiquantitive manner by two pathologists independently. Firstly, the slides (both H&E and EVG stained slides) were examined by a senior resident pathologist (evaluator 1) and then by a senior consultant pathologist (evaluator 2). All discordant observations were re-evaluated and agreed upon, and a final grade was given (table 2). The revealed grades of all cases were then further
subcategorized into low elastosis (Grade 0, 1 and 2) and high elastosis (Grade3). Elastosis grading was done blindly of other features.

**Table 1:** Grading system of elastosis using H&E and EVG stain

| Grade | Description |
|-------|-------------|
| Absent | No elastosis is detected |
| Grade 1 | Small deposits (single elastin fibrils or a thin rim of elastosis around ducts) were present |
| Grade 2 | Thicker zones of elastosis were found |
| Grade 3 | Large deposits dominated substantial areas of the tumor |

**Table 2:** The discrepant cases between evaluators and the final agreed grade

| No. | Discrepant cases | Final Grade |
|-----|------------------|-------------|
|     | Evaluator 1 | Evaluator 2 |              |
| 1   | Grade 0    | Grade 1    | Grade 1     |
| 2   | Grade 1    | Grade 0    | Grade 1     |
| 3   | Grade 0    | Grade 2    | Grade 1     |
| 4   | Grade 1    | Grade 0    | Grade 0     |
| 5   | Grade 1    | Grade 0    | Grade 1     |
| 6   | Grade 1    | Grade 2    | Grade 1     |
| 7   | Grade 2    | Grade 1    | Grade 1     |
| 8   | Grade 2    | Grade 3    | Grade 3     |
| 9   | Grade 1    | Grade 3    | Grade 3     |
| 10  | Grade 0    | Grade 1    | Grade 1     |
| 11  | Grade 2    | Grade 1    | Grade 1     |
| 12  | Grade 3    | Grade 2    | Grade 2     |
| 13  | Grade 1    | Grade 0    | Grade 1     |

The collected information was entered using Epi-data program and then transferred to SPSS-24 for analysis. Associations between different categorical variables were assessed by Chi Square test. In addition, univariate survival analyses of time to death due to breast cancer (disease specific survival) was performed using the Kaplan–Meier survival curves with log-
rank test for comparisons. Two-sided p-values of <0.05 was considered statistically significant. This study was ethically approved by the Histopathology Department of the AFH.

**Results**

A total of 80 cases, including 40 biopsies and 40 resection specimens were included. Patient’s age ranged from 27 years to 83 years, with mean age of 51.7 years ±13.3 with median of 52.5, minimum of 27 and maximum of 80. Among the total sample, 80% (n=64) were diagnosed with invasive ductal carcinoma (NOS), while 12.6% (n=10) of the cases were diagnosed with infiltrating lobular carcinoma. The remaining (n=6, 7.5%) cases were tubular, medullary and mucinous carcinomas. Postmenopausal cases in this study accounted for 52.5%. More than half of the cases were graded as Nottingham grade 2 (n=41, 51.3%), while grade 1 and grade 3 constituted 23.8% (n=19) and 25% (n= 20) respectively. Throughout the study period, the follow-up of patients ranged from 0.03 years (3 months) to 10 years with mean of 43 months ± 28.3 months and median of 33.0 months, minimum of 3 and a maximum of 120 months.

Using EVG, absent elastosis, grade 1, grade 2, and grade 3 were observed in 12.5%, 37.5%, 30%, and 20% respectively. Elastosis grading on H&E conducted by the two pathologists independently revealed strong inter observer agreement (Kappa coefficient is 0.858). Eleven cases out of 13 cases were agreed on high elastosis (84.6%) and 66 out of 67 cases were agreed to be low elastosis (98.5%) by the two assessors.

A statistically significant relation between high elastosis and ER positivity was observed (P 0.015). In this regard, 60.9% of low elastosis cases were ER positive compared to 93.6% of high elastosis cases were ER positive. Among low elastosis cases, 50% were Her-2 negative compared to 81.2% among high elastosis. This difference was statistically significant (p-value is 0.045). On the other hand, no statistically significant relationship between elastosis and other entities including menopausal status, tumor type and grade, PR and Ki6 was observed.

The overall 5-year survival in the study sample was 77.9%. High elastosis patients didn’t show any advantage for the overall survival rate compared to low elastosis patients (p-value 0.5). Comparison between the survival curves among high and low elastosis groups is shown in figure 2, which shows almost similar survival rate until the 5th year of follow-up. In
addition, there was no difference between the two groups in relation to clinical remission (62.5% vs. 68.8% in low and high elastosis groups respectively, p value 0.5).

In relation to the diagnostic accuracy of H&E stain in quantifying elastosis, table 3 illustrates the 2x2 table of H&E compared to EVG stain methods in quantifying elastosis. The sensitivity and specificity of H&E stained method compared to EVG stain method (the gold standard) were 68.75% and 96.88% respectively. The positive predictive value was 84.62%, negative predictive value was 92.54%, the positive likelihood ratio was 22 and the negative likelihood ratio was 0.32.

**Figure 2**: Survival among high and low elastosis groups

**Table 3**: 2 x 2 table of H&E compared to EVG stain methods in quantifying elastosis.

|                  | EVG stain   |           | Total |
|------------------|-------------|-----------|-------|
|                  | Low elastosis | High elastosis |     |
| H&E stain        | 62          | 5         | 67    |
|                  | 2           | 11        | 13    |
| Total            | 64          | 16        | 80    |

**Discussion**

This is the first study addressing the trend of elastosis among breast cancer patients in Oman, and addressing the diagnostic accuracy of H&E stain in quantifying elastosis worldwide. This study showed the common occurrence of elastosis among local patients, and emphasized the
significant association between elastosis in breast cancer and estrogen positivity. In addition, this study showed good diagnostic value for H&E stain in quantifying elastosis.

The amount of elastic fibers in breast tissue and especially in malignant cases is an indicator of good prognosis in such patients. This has been evaluated in previous studies which showed different results. For example, in a study done by Bindu S et al (2016), it was found that the trend of elastic tissue content among 66 primary breast carcinomas was different compared to our figures. They observed that 9.09% of cases showed absent or grade 1 elastosis, 83.3% of cases presented with grade 2 elastosis, and 7.5% had massive elastosis (grade 3). This variation in the occurrence of elastosis in different population can be explained by differences in ethnicity and genetics, and differences in the occurrence of various subtypes of breast cancer studied the relative low number of ducts in the circumscribed and expansible tumors that displace these ducts. In addition, it differs depending on the degree of anaplasia; being more in the well-differentiated malignant lesions compared to the poorly-differentiated malignant lesions. Moreover, elastosis occurrence differs significantly in cases not yet treated compared to cases examined after treatment.

With regard to the relationship between elastosis and steroid receptors, Salesse S et al. (2018) observed that large amount of elastin is associated with intense estrogen positivity. However, this relation is noticed in a lesser degree with the PR. Similar results were observed in different studies as well as the present study.

Many studies have observed the association between Her2 and ki67 and elastosis. In this regard, Chen Y et. Al (2014) found that stromal elastosis is almost always associated with factors that give good prognosis including Her2 negativity and low Ki67. In this study, similar results between elastosis and Her2 negativity were observed; however, no association between elastosis and ki67 in this was observed conflicting with other studies for Ki67.

The value of elastosis in survival has been investigated in other studies and the results have been contradictory. Some studies found that elastosis in breast cancer is associated with better prognosis and prolonged survival. This may be explained as tumors with no elastosis found to be more aggressive and associated with tumor necrosis and lymph node metastasis and vascular invasion. This finding was explained in a study as elastosis is probably not a favorable microenvironment for tumor growth and spread.
The amount and the extent of elastosis in the stroma within the tumor in this study show to have little value in disease-specific survival or prognosis. This is consistent with other studies, including a study which showed that there was a somehow a better survival in patients with extensive elastosis when compared with those who had none, but that difference was so small as to be of little consequence to the overall duration of survival.\(^4\)

In relation to the diagnostic accuracy of H&E stain in quantifying elastosis, H&E is considered the primary stain and the most commonly used staining method for histology slides.\(^7\) It is by far preferred by pathologists for viewing different cellular and various tissue structural details. It contains the two dyes hematoxylin and eosin. Hematoxylin gives the acidic (or basophilic) structures a purplish blue color while eosin stains basic (or acidophilic) structures red or pink.\(^7,8\) Elastic fibers can be recognized in standard H&E slides as a deposit of grey, fibrillar material.\(^2,7\) In contrast, EVG gives these fibers black color which explains a higher sensitivity than the visual recognition of the grey material in H&E slides.\(^2\) However, due to the high sensitivity of H&E, only small proportion of H&E slides with absent elastosis were in fact grade 1 by EVG, and likewise for H&E grade 1 being grade 2 in EVG. However, there was almost complete concordance between H&E and EVG in quantifying grade 3 cases.

**Conclusion**

This study is the first study addressing elastosis among Omani breast cancer patients and addressing accuracy of H&E stain in quantifying elastosis. Elastosis occurrence varies in different breast cancer populations. The strong relation between high elastosis and estrogen positivity and negative HER2/neu receptor among breast cancer patients was emphesized, hence the presence of elastosis can be considered as a surrogate marker for estrogen positivity in the initial screening procedure. In addition, H&E stain is considered as an accurate method for quantifying elastosis compared to EVG staining method.

**Conflict of interest**

No conflicts of interests were involved in this work.

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