Clinicopathologic update of calcium oxalate in breast:
A 15-year retrospective review

Koorosh Haghayeghi MD, PharmD1 | Mehran Najibi MD1 | Hai Wang MD1 | Linda Donegan MD2 | Yihong Wang MD, PhD1

1Department of Pathology and Laboratory Medicine, Rhode Island Hospital and Lifespan Medical Center, Warren Alpert Medical School of Brown University, Providence, RI, USA
2Department of Diagnostic Imaging, Rhode Island Hospital and Lifespan Medical Center, Warren Alpert Medical School of Brown University, Providence, RI, USA

Abstract
Mammary malignancies are radiologically detected by presence of masses, architectural distortions or microcalcifications. Unlike calcium hydroxyapatite, calcium oxalate (CaOx) deposits have been almost exclusively associated with benign mammary processes. The etiology and mechanism of mammary CaOx deposition remains poorly understood, and the original studies elucidating its histopathologic correlation are dated several decades ago. We reviewed radiopathologic findings of breast biopsies and excisions to re-examine the clinicopathologic significance of CaOx deposits and to ascertain potential radiologic characteristics for their identification. Fifty patients from 2004 to 2019 with reported “calcium oxalate” were retrospectively reviewed. CaOx was invariably detected with histopathologic changes of nonproliferative ducts/cysts (90%, 45 of 50), and less commonly, ducts/cysts with usual ductal hyperplasia (10%, 5 of 50). CaOx was missed on one biopsy with a subsequent excision showing apocrine cyst with CaOx. Despite the benign pathological findings, mammographic findings corresponding to CaOx ranged from benign to highly suspicious with 20% categorized as benign (round or punctuate), 22% as intermediate amorphous, 14% as suspicious (coarse/heterogeneous), and 18% as highly suspicious/pleomorphic, respectively. Lobular carcinoma in situ (LCIS) was present in separate fields from CaOx containing benign ducts in two cases which were radiologically characterized as “grouped heterogeneous” and “localized linear.” On imaging, more than half of the cases (52.5%) had a corresponding BI-RADS score of 4 and the calcifications were associated with variable distributions and appearances. In conclusion, this is one of the largest studies of CaOx in breast with radiology and pathology correlation. The radiologic appearances of CaOx are nonspecific from benign to highly suspicious. Identification of CaOx on the biopsy is reassuring for a benign diagnosis. Incidental atypical lesions can occur that are often not directly associated with CaOx. CaOx may be overlooked on pathologic evaluation which results in unnecessary surgery. Our findings support close radiologic–pathologic correlation for clinical decision-making pertaining to breast calcifications.

Keywords
breast, calcifications, calcium oxalate, microcalcification
Mammary calcifications are broadly classified into macro- and microcalcifications. The underlying pathogenesis of macro-calcifications is due to non-neoplastic processes including inflammation or trauma while microcalcifications signify cellular turnover with a potential for malignancy.\textsuperscript{1} Nearly one third of all breast malignancies are associated with microcalcifications, and half of nonpalpable mammary cancers are detected by suspicious radiologic microcalcifications.\textsuperscript{2-3} The vast majority of microcalcifications have a benign course, but their presence may be the only initial sign of breast cancer prior to forming any discernable masses.\textsuperscript{4} DCIS is a nonobligate precursor of infiltrating ductal carcinoma. Nearly 90\% of women with DCIS have suspicious microcalcifications on mammography, and the disease comprises up to 50\% of all mammographically detected breast cancers.\textsuperscript{5-8}

Microcalcifications can be grouped according to morphology, distribution, or location.\textsuperscript{1} Round or punctate microcalcifications classically arise in the lumen of lobular acini. When exceeding 0.5 mm they are called round, while below 0.5 mm findings are referred as punctuate. Both are generally placed in the "typically benign" category. Intermediate amorphous microcalcifications are 200-300 µm. They are found in association with benign fibrocystic change or noncomedo DCIS. Because the amorphous form of calcification occurs in both benign and malignant lesions, further investigation is often warranted. Studies by Berg et al have demonstrated that 40\% of amorphous microcalcifications are malignant and therefore warrant biopsy.\textsuperscript{5} Coarse heterogeneous forms refer to microcalcifications exceeding 0.5 mm and are easily visible, irregular and coalesce. Pleomorphic calcifications can be fine, linear, or linear breached; they correspond to calcifications whose size is under 0.5 mm and are more visible than other types.\textsuperscript{1} They are categorized as suspicious and are classified BI-RADS 4, or even BI-RADS 5 when the distribution is in a segmental pattern. In a meta-analysis on 40 publications and 10 665 cases of microcalcifications, Rominger and colleagues reported a global rate of malignancy of 9\% for round and punctuate, 27\% for amorphous, 13\% for coarse heterogeneous and 78\% for pleomorphic calcifications.\textsuperscript{10} Evidence of microcalcification clusters in association with pleomorphism, branching, architectural distortion, or any mass or density, increases the predictive value for malignant processes.\textsuperscript{11} Biochemically, they are classified as two distinct types with different implications. Type I microcalcifications are comprised of calcium oxalate (CaOx).\textsuperscript{12} These refractile and birefringent crystals are often difficult to detect under a nonpolarized light and are believed to be associated with apocrine cysts.\textsuperscript{13} Type II microcalcifications are more common and may be associated with either benign or malignant lesions. These are comprised of calcium phosphate with nonbirefringent and refractile properties that stain purple on hematoxylin.\textsuperscript{14} Both types can co-exist and their components cannot be determined by mammography.\textsuperscript{15} In biopsies in which type II microcalcifications are not identified, examination of sections under polarized light usually reveals the
presence of CaOx crystals. Different specimen processing procedures may, however, interfere with detection of these crystals. While calcium phosphate may be caused by cellular degeneration or necrosis due to a possible underlying malignancy, it has been long hypothesized that calcium oxalates are products of secretions and are therefore benign. However, CaOx cannot be metabolized by breast epithelial cells and the emerging evidence suggests that long lasting oxalate exposure may trigger cellular and genetic changes.

Oxalate is an abundant organic acid found in nature. It is synthesized through incomplete oxidation of carbohydrates in plants and has a high affinity for binding divalent cations including calcium. CaOx is a plant bio-mineral with a role of maintaining low cytosolic calcium concentration for homeostasis. CaOx mineralization has no known physiologic role in vertebrates. While exogenous intake of oxalate has known implications in urinary stone formation, endogenous biosynthesis of CaOx also takes place.

The immediate precursor of oxalate in mammalian cell is glyoxylate. Peroxisomes and mitochondria are two major sites of production of endogenous glyoxylate. In the peroxisomes, glyoxylate can be derived from glycine through the enzymatic function of hydroxycacid oxidase 1 (HAO1). Excess glyoxylate in peroxisomes is then converted to either glycolate or oxalate by glyoxylate oxidase (GO). Mitochondrial glyoxylate is derived from 4-hydroxy-proline which is ultimately converted to glyoxylate through the action of 4-hydroxy-2-ketoglutarate lyase (HKGA) and is then transported into the cytoplasm, where it is converted to oxalate by lactate dehydrogenase (LDH).

Evaluation of breast microcalcifications is a major clinical intervention in diagnosis and management of breast cancer. Historically, only type II microcalcifications have had clinical significance, while type I calcifications were largely attributed to indolent changes of the breast, except uncommon association with LCIS and rarely with DCIS. In one of the largest histologic-radiologic studies on mammary CaOx, Frouge et al evaluated clinicopathologic findings of oxalate in 42 cases and reported the majority of CaOx lesions of benign origin (n = 37), followed by DCIS (n = 2), IDC (n = 2), and LCIS (n = 1). It is unclear whether these previously reported rare associations of CaOx with atypical proliferative lesions were incidental concurrent findings in the background or that the crystals were directly deposited in the premalignant proliferative processes. Our retrospective study of CaOx deposits in breast biopsy and excision specimen re-enforces the benign histopathologic nature of CaOx with summary of radiology-pathology correlations.

2 | METHODS

With Institutional Review Board approval, a retrospective natural language search of the pathology database (CoPath) was performed to identify all patients who underwent breast biopsy and excision at one of the three hospitals within our health care system.
between 2004 and 2019. The search was constructed to retrieve any cases carrying a diagnostic description for "calcium oxalate." Fifty-one cases were identified. Histologic sections were reviewed by YW, KH and HW and assessed to determine whether they met criteria for inclusion, specifically to confirm the presence of CaOx as the targeted lesion. Patients' radiologic findings, including BI-RADS scores and microcalcification subtypes, were populated.

All biopsies were stereotactic-guided and done with a 9 gauge vacuum-assisted biopsy device.

Results of the biopsy, subsequent surgical excision, follow-up imaging, and clinical course were recorded along with patient demographic information.

3 | RESULTS

The study included 51 specimens from 50 patients: 44 were stereotactic biopsies and 7 were excisions.

The median age was 49 years old (range 37-76). Patients with CaOx findings had a range of radiologic findings of the microcalcifications: 20% (10/50) were radiologically categorized as benign (round or punctuate), 22% (11/50) as intermediate amorphous, 14% (7/50) as suspicious (coarse/heterogeneous), 18% (9/50) as highly suspicious/pleomorphic, and the remainder 26% (13/50) had no detailed radiologic descriptions of the characteristics of the microcalcifications (Figure 2). Architectural asymmetry and enhancing mass/density contributed to only 4% (2/50) and 2% (1/50) of the cases with oxalate findings, respectively. CaOx deposits were most commonly detected in nonproliferative breast tissue including dilated cysts (42%, 21/50), apocrine change (26%, 13/50), and benign ducts (14%, 8/50). In proliferative instances, usual ductal hyperplasia was the only culprit (10%, 5/50) (Table 1). In rare instances, CaOx crystals were found in chronic granulomatous inflammation (2%, 1/50), intraductal papilloma (2%, 1/50), columnar cell change (2%, 1/50), and stromal tissue (2%, 1/50) (Figure 3).

CaOx deposition was noted only in indirect association with proliferative cells with atypia such as LCIS (4%, 2/50). No CaOx was detected in DCIS, invasive carcinoma, pseudoangiomatous stromal hyperplasia (PASH), collagenous spherulosis, or fibroadenomatous change. The most common histopathologic changes seen in the vicinity of CaOx were UDH, followed by columnar cell change, fibrocystic change/duct ectasia, and PASH/collagenous spherulosis (Table 1).

FIGURE 3 Histopathologic changes seen in association with calcium oxalate deposits. A, Fibrocystic change (H&E, 200×), B, papilloma, (H&E, 200×), C, usual ductal hyperplasia (H&E, 200×), D, columnar cell change with apocrine features (H&E, 200×), and E, chronic granulomatous disease (H&E, 100×). F, Benign duct with surrounding inflammation and apocrine metaplasia (H&E, 100×).
Excision was performed in 7 cases due to radiologic evidence of a mass (n = 1), LCIS (n = 2), DCIS (n = 1), UDH (n = 1), radial scar (n = 1), and discordant absence of calcifications on initial biopsy (n = 1) (Table 1). The excision of the aforementioned biopsy lacking overt microcalcifications showed a complex sclerosing lesion with radial scars, nodular sclerosing adenosis, papillomas with florid ductal hyperplasia, with CaOx identified in association with a benign cyst/microcyst. No upgrade was identified in excision specimens.

In the case of excision for the enhancing mass, the main histologic finding was ductal ectasia and usual ductal hyperplasia, with CaOx seen in apocrine cysts.

Both cases associated with LCIS diagnosis pertained to a 64-year-old woman with first needle biopsy revealing focal CaOx within a duct along with a separate field showing a focus of LCIS. The subsequent excision revealed LCIS with pagetoid spread along ducts. CaOx deposits were associated with benign ducts and were not deposited in any foci with LCIS.

The excision performed on DCIS was a 45-year-old woman. DCIS was associated with calcium phosphate on needle biopsy, and CaOx was in benign ducts with fibrocystic change. One excision was performed due to radiopathology discordance in a 51-year-old woman with no calcification found on the needle biopsy. Excision identified polarizable CaOx deposit with benign breast changes including UDH and microcyst. Follow-up data were available for 34 patients, all with negative findings. The mean follow-up interval was 60 months; the median was 45 months (range 5-179 months). (Table 1).

Limitations of our study include the retrospective design, which precludes complete follow-up on all patients. As the cases were primarily selected by keyword search for “calcium oxalate,” cases with unspecified diagnosis for calcifications were not included in our study.

### 4 | CONCLUSION

CaOx type of microcalcification is associated with variable radiologic findings which range from completely benign to highly suspicious. Biopsy confirmation is therefore critical in appropriate assessment for malignancy. Identification of CaOx on the biopsy is reassuring for benign processes. Incidental atypical lesions can occur that are often not associated with CaOx crystals. CaOx may be overlooked in pathologic examination and careful examination is needed to avoid unnecessary surgical procedures. Our retrospective study of CaOx and long-term follow-up did not show any increased rate of proliferative processes in patients with a history of calcium oxalate. Therefore, close radiologic-pathologic correlation is critical for clinical decision-making pertaining to breast calcifications.

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### CONFLICT OF INTEREST

The authors declare no conflicts of interest pertaining this study.

### DISCLOSURES

The authors of this study have no financial/commercial relationships to declare. This study has partly been presented at the College of American Pathologist 2017 Annual Meeting.

### ORCID

Koorosh Haghayeghi https://orcid.org/0000-0003-1527-1025

Yihong Wang https://orcid.org/0000-0003-1252-5579

### REFERENCES


