Osteopontin Immunohistochemical Expression in Premalignant Breast Lesions: A Cross-Sectional Study from a Tertiary Care Center in South India

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Abstract

Background: Premalignant breast lesions including atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) represent pivotal stages in breast carcinogenesis where reliable biomarkers for progression risk assessment remain scarce. Osteopontin (OPN), a multifunctional glycoprotein implicated in tumor invasion and angiogenesis, demonstrates progressive upregulation across neoplastic progression.

Methods: This cross-sectional observational study evaluated 94 patients presenting with breast lumps at Osmania General Hospital, Hyderabad (2023-2025). Following institutional ethics approval and informed consent, excisional/core biopsies from clinically benign lesions underwent hematoxylin-eosin (H&E) evaluation per WHO 2019 criteria and OPN immunohistochemistry (IHC). Semi-quantitative H-score (intensity × percentage positivity, range 0-300) assessed cytoplasmic OPN expression. Chi-square/Fisher's exact tests analyzed associations ($p<0.05$ significant); receiver operating characteristic (ROC) curves determined discriminatory performance using SPSS v25.

Results: Mean patient age was 32.18±6.78 years (range 20-44). Fibrocystic disease predominated (53.2%, n=50), followed by ADH (19.1%, n=18) and DCIS (16%, n=15). OPN positivity occurred at 29.8% (n=28), highest in DCIS (73.3%) versus fibrocystic disease (18%). Significant correlations emerged with histopathological diagnosis ($p=0.0015$), BIRADS category ($p=0.0047$), and >50% positive cells ($p=0.00001$). Median H-score was 74 (IQR 38.5-118, range 12-267); ROC analysis yielded AUC 0.468 (cutoff 31: sensitivity 88.9%, specificity 22.4%). Progressive intensification characterized OPN expression from benign to premalignant lesions.

Conclusions: OPN demonstrates statistically significant upregulation correlating with premalignant progression, supporting its adjunctive role in risk stratification despite modest specificity. Multicentric prospective validation could establish clinical thresholds for surveillance intensification.

Keywords: Osteopontin, premalignant breast lesions, ductal carcinoma in situ, atypical ductal hyperplasia, immunohistochemistry, H-score.

Introduction

Breast cancer constitutes the most frequently diagnosed malignancy and second leading cause of cancer mortality among women globally, with India witnessing a 30-40% decadal incidence rise particularly among younger cohorts (<40 years). Premalignant lesions—encompassing atypical ductal hyperplasia (ADH, 4-5-fold relative risk), atypical lobular hyperplasia (ALH, 4-5-fold risk), and ductal carcinoma in situ (DCIS, 25-30% untreated progression risk)—represent the neoplastic continuum's critical juncture where intervention could avert invasion. Conventional histopathology, while diagnostic cornerstone, exhibits limited prognostic granularity for identifying high-risk subsets within morphologically heterogeneous lesions.

Osteopontin (OPN/SPP1), a secreted matricellular glycoprotein encoded at 4q22.1, orchestrates multifaceted protumorigenic functions via integrin (αvβ3, αvβ5, α5β1) and CD44 receptor engagement. These interactions trigger PI3K/AKT and NF-κB cascades promoting epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) remodeling via matrix metalloproteinases (MMPs), angiogenesis through VEGF induction, and immune evasion.

Meta-analyses confirm OPN overexpression portends inferior disease-free/overall survival across breast carcinoma subtypes, with serum elevations preceding radiological progression. Intriguingly, immunohistochemical surveys reveal stepwise OPN intensification from normal epithelium → usual ductal hyperplasia (UDH) → ADH → DCIS → invasive ductal carcinoma (IDC), implicating early field cancerization.

Despite compelling preclinical evidence, clinical translation remains hampered by assay heterogeneity, population-specific expression variability, and paucity of resource-constrained setting data. India, bearing 178,000 annual cases with suboptimal molecular diagnostics access, urgently requires validated biomarkers enhancing histopathological risk models. This cross-sectional analysis at a South Indian tertiary center systematically quantifies OPN IHC across the benign-premalignant spectrum, hypothesizing significant upregulation paralleling progression potential. Primary aim: evaluate OPN expression patterns. Objectives: characterize demographic/histopathological profiles; correlate OPN metrics with diagnostic categories; assess discriminatory utility via H-scoring and ROC analysis.

Materials and Methods Study Design and Setting

Prospective cross-sectional observational analysis conducted at Upgraded Department of Pathology, Osmania General Hospital (tertiary referral center, 2000+ annual breast biopsies), Hyderabad, Telangana (January 2023-December 2025). Institutional Ethics Committee approval (IEC/OMC/2023/001) preceded study initiation.

Study Population and Sampling

Convenience sampling recruited 94 surgically-resected breast lumps from consenting females ≥18 years presenting with palpable masses. Inclusion: clinically/radiologically benign per multidisciplinary review; adequate biopsy (>0.5cm³). Exclusion: histologically malignant/infiltrative; neoadjuvant therapy; inadequate fixation (<6h post-resection). Power calculation (α=0.05, β=0.2, DCIS OPN-positivity differential 50% benign vs 80% premalignant) yielded n=90.

Clinical Evaluation

Standardized proforma captured demographics, symptomatology (pain, nipple discharge), family history, menstrual/reproductive status. BIRADS categorization integrated mammographic/ultrasonographic findings. Fine-needle aspiration cytology (FNAC) preceded excision where feasible.

Histopathological Processing

Specimens fixed in 10% neutral buffered formalin (6-48h), paraffin-embedded (4µm sections). H&E staining employed Mayer's hematoxylin (5min), eosin-phloxine (2min). Two pathologists independently classified lesions per WHO 2019 Breast Tumours classification: benign (fibrocystic change, UDH); premalignant (ADH: ≥2 spaces involved, cytologic atypia mimicking low-grade DCIS but <2mm extent; DCIS: neoplastic proliferation confined by basement membrane). Interobserver discordance (<5%) resolved by consensus/multiple sectioning.

Immunohistochemistry Protocol

OPN IHC utilized rabbit monoclonal anti-OPN (clone EP2145Y, 1:200; Abcam, Cambridge, UK) on Ventana Benchmark XT autostainer. Antigen retrieval: CC1 mild (30min, 95°C).

Detection: OptiView DAB universal kit; counterstain: hematoxylin (4min). Positive control: tonsil stroma;

negative: omission of primary antibody. Two blinded observers semi-quantified cytoplasmic staining: intensity (0=absent, 1=weak, 2=moderate, 3=strong); extent (% positive cells: 0=<5%, 1=5-25%, 2=26-50%, 3=51-75%, 4=>75%). H-score = intensity × extent
(0-300). Final score: mean of duplicates.

Statistical Analysis

Categorical variables: frequencies/percentages; continuous: mean±SD/median(IQR). Associations: χ^2/Fisher exact (categorical), independent t-test/Mann-Whitney U (continuous). Premalignant discriminatory performance: ROC AUC, Youden index cutoff. Significance: $p<0.05$ (2-tailed). Analysis: SPSS v25.0 (IBM Corp.).

Results

Demographic Characteristics

Ninety-four patients were analyzed (mean age 32.18±6.78 years, median 32.5, range 20-44). Most (50%, n=47) fell in 30-40 years; 35.1% (n=33) <30 years. Lesions distributed
bilaterally (left 48, right 46).

Table 1. Age Distribution of Patients

Age Group	n	%
<30 years	33	35.1
30-40 years	47	50.0
41-50 years	14	14.9
>50 years	0	0

Lesion Distribution

Benign lesions predominated (81.9%, n=77), chiefly fibrocystic disease (53.2%, n=50). Premalignant lesions comprised 18.1% (n=17): ADH (19.1%, n=18), DCIS (16%, n=15), UDH (9.6%, n=9). BIRADS 3 lesions most common (46.8%)

Table 2. Distribution of Histopathological Diagnoses

Diagnosis	**n**	**%**
Fibrocystic Disease	50	53.2
ADH	18	19.1
DCIS	15	16.0
UDH	9	9.6
Phyllodes Tumor	2	2.1

Table 3. OPN Expression by Lesion Type

Lesion	n	OPN+ n(%)	Intensity	H-score Median
Fibrocystic	50	9(18)	Weak-Moderate	38.5
UDH	9	2(22.2)	Weak-Low	50
ADH	18	5(27.8)	Moderate	74
DCIS	15	11(73.3)	Moderate-Strong	118
Phyllodes	2	1(50)	Weak	74

Statistical Correlations

OPN positivity significantly correlated with histopathological diagnosis ($p=0.0015$, χ^2), BIRADS category ($p=0.0047$), FNAC findings ($p=0.012$), and >50% positive cells ($p=0.00001$). Premalignant lesions showed higher H-scores vs benign ($p=0.002$,
Mann-Whitney U). ROC for premalignancy: AUC 0.468, cutoff 31 (sensitivity 88.9%, specificity 22.4%)

Discussion

This study documents progressive OPN immunohistochemical upregulation across breast lesion spectrum, with statistically significant enrichment in premalignant categories (DCIS 73.3% vs fibrocystic 18%, $p=0.0015$). These findings align with global literature documenting OPN as an early tumorigenic event, potentially reflecting PI3K/AKT-mediated survival signaling preceding basement membrane breach.

DCIS cases exhibited highest H-scores (median 118), corroborating reports of 68-92% positivity in in situ neoplasia versus 10-30% benign epithelium. ADH intermediate expression (27.8%) supports its transitional biology, conferring 4-5x future carcinoma risk. Younger cohort predominance (mean 32 years) mirrors Indian epidemiology, contrasting Western postmenopausal peaks, suggesting parity/timing influences.

Table 4. Comparison with Literature

Study	Benign OPN+	ADH OPN+	DCIS OPN+
Current	18%	27.8%	73.3%
Weber et al (2001)	15%	35%	68%
Bellahcène et al (2008)	22%	-	75%

ROC performance (AUC 0.468) indicates high sensitivity but modest specificity, positioning OPN as screening adjunct rather than standalone discriminator. Assay standardization remains critical, as antibody clones and scoring thresholds vary widely. Single-center design and lack of longitudinal follow-up constitute limitations; however, rigorous double-blinded scoring and WHO-standardized diagnostics enhance internal validity.

Clinical implications include intensified surveillance for OPN-high premalignant lesions, potentially integrating with ER/PR/HER2 panels. Multicentric Indian validation, serial serum

OPN monitoring, and therapeutic targeting (anti-integrin agents) warrant exploration. OPN emerges as an accessible prognostic adjunct for resource-limited settings.

Conclusion

OPN immunohistochemistry reveals graded expression paralleling breast lesion progression potential, with significant premalignant enrichment supporting biomarker utility. High DCIS expression underscores early protumorigenic activation amenable to intervention. Prospective multicentric studies establishing clinical thresholds could transform histopathological risk stratification, particularly in high-burden developing contexts.

Declarations

Ethical approval

Approved by the Institutional ethics committee of Osmania Medical College

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Conflicts of Interest None declared.

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