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Efficacy of immunohistochemistry in diagnosing papillary lesions of the breast

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Abstract
Introduction: Papillary neoplasms of breast form a heterogeneous group of diseases, spanning a spectrum of benign, atypical, and malignant lesions constituting less than 10% of benign lesions and less than 1% of breast cancers.
Aim of the study: To differentiate and determine the behaviour of papillary lesions of the breast based on immunohistochemical staining pattern by CD10, Smooth Muscle Actin and p63.
Materials and Methods: A total of 15 cases of papillary lesions which included intraductal papillomas, papillary epithelial hyperplasia, papillary carcinomas, micropapillary ductal carcinoma in situ [DCIS] were evaluated by CD10, SMA and p63 immunostains.
Results: Out of the 15 cases, 5 diagnosed as duct papillomas showed positivity for all the three markers. 6 cases diagnosed as papillary carcinomas were uniformly negative for all stains. 4 cases diagnosed as papillary epithelial hyperplasia showed discontinuous variable staining with p63, making interpretation of an intact myoepithelial cell layer difficult. The lack of a basal myoepithelial cell layer in these cases suggests progression to invasive disease and explains distant metastases from previously reported "intraductal papillary carcinoma."
Conclusion: It is prudent to use p63, which is a nuclear marker and does not show cross reactivity with stroma, proving to be better in differentiating the papillary lesions of breast unlike CD10 and SMA which stain the cytoplasm of myoepithelial cells and show cross reactivity with the mammary stroma.

Keywords: Papillary lesions of breast, IHC breast lesions, p63, SMA, CD10 in breast lesions.

Introduction

Papillary lesions of the breast are a spectrum of diseases which include benign, atypical, and malignant diseases [1]. Sub classification of these lesions may be diagnostically challenging many a times, especially when the sample contains limited amount of tissue as in the core needle biopsies unlike excisional biopsies which give a greater amount making the diagnosis easier to some extent [2]. Correct diagnosis is not only crucial, but also difficult, as most of the benign and malignant papillary lesions have almost similar morphology.

Papillary lesions of the breast form a heterogeneous group constituting about 10% of all the benign and less than 1% of malignant lesions of the breast [3]. Having varied morphologic features that carry varying prognostic implications for affected patients, the spectrum of papillary lesions of the breast include:

1. Benign papilloma
2. Papilloma with atypical ductal hyperplasia (ADH)
3. Papillary carcinoma in situ
4. Invasive papillary carcinoma

These neoplasms are united by ''papillary'' morphology, consisting of arborizing fronds with a central fibrovascular core which is mostly derived from the wall of the ducts within the breast [4]. The ducts are frequently dilated, with protrusion of the fibrovascular cores into the ductal lumen of various caliber and the lining epithelium may be hyperplastic or malignant. These are mostly benign, but pre-malignant (atypical) and malignant histological features can also be identified including papillary ductal carcinoma in situ (DCIS), intracystic papillary carcinoma, and solid papillary carcinoma [1, 2, 3].

Identification of a myoepithelial cell (MEC) layer histologically or by immunohistochemical analysis is a key feature in distinguishing benign from malignant lesions. This can be challenging in case of core needle biopsies and also in cases with epithelial hyperplasia, atypical ductal hyperplasia (ADH), sclerosis and atypia [4].

Distinction of these lesions on routine hemtoxylin and eosin (H & E) sections can sometimes be difficult, for which IHC is useful in delineating the myoepithelial cell layer [5]. Several immunohistochemical stains, like CD10, SMA, and p63, have been used successfully to identify myoepithelial cell layer. CD10 and SMA are cytoplasmic stains, while p63 is nuclear stain for MEC. However, CD 10 and SMA also stain vascular smooth muscle cells and myofibroblasts making interpretation difficult in fibroblastic stroma or if the vessels are in proximity to the epithelium. p63, a member of the p53 gene family, shows no cross-reactivity with myofibroblasts or vascular smooth muscle. This study is therefore being done to evaluate the efficacy of IHC markers CD10, SMA and p63 in diagnosing the various papillary lesions of the breast [5].

Aim of the study

To differentiate and determine the behaviour of papillary lesions of the breast based on immunohistochemical staining pattern by CD10, Smooth Muscle Actin (SMA) and p63.

Materials and Methods

The present study is a prospective study conducted over a period of one year from June 2017 to May 2018. A total of 324 breast biopsies received from the departments of General Surgery and Oncology at Kamineni Academy of Medical Sciences and Research Centre, Hyderabad were examined. The biopsies included both excisional biopsies and core needle biopsies. Out of these, 15 cases turned out to be Papillary lesions of the breast. Tissue specimens of these fifteen cases were focussed in this study. They were fixed routinely in 10% formalin and embedded in paraffin, and sections were stained with H&E using conventional methods. All the 15 cases were reviewed by 2 pathologists for confirmation of diagnosis and for selection of blocks for Immunohistochemical staining.

Immunohistochemical Analysis

Deparaffinized 4- to 5-µm sections of 1 block from each case were rehydrated and subjected to heat-induced epitope retrieval procedures optimized for each antibody. All 3 antibodies, CD10 (dilution 1:400; Path insitu), SMA (dilution 1:100; Path insitu), and p63 (dilution 1:1500; Path insitu) were applied to sequential sections from each block. A standard avidin biotin immunoperoxidase technique was used. Sections were counterstained with 0.1% methyl green. In addition to a separate positive control sample of normal breast tissue that was run with each stain, internal positive control samples were present in every case in the form of nonneoplastic breast tissue. Negative control samples using 10% bovine serum albumin in place of the primary antibody were run with each batch of stains as well.

Observations and Results

Of the 324 cases studied, 15 lesions were diagnosed as papillary lesions which ranged from benign ductal papillomas to malignant papillary carcinomas including papillary epithelial hyperplasia. Out of the 15 cases, 05 cases were ductal papillomas (Fig 1), 04 cases were of papillary epithelial hyperplasia (Fig 2) and 06 cases were of papillary carcinoma (Fig 3). This group of benign papillomas (Fig 1a,1b,1c, 1d) and papillary epithelial hyperplasias (Fig 2a, 2b, 2c, 2d) had a distinct, easily recognizable MEC layer on H&E-stained sections. CD10, SMA and p63 all showed positive staining in these lesions. There was a diffuse, continuous staining of the peripheral rim with CD10 and SMA in the majority of cases with a few skip areas seen in occasional cases. The nuclear stain p63 showed irregularly spaced nuclear staining with occasional gaps. Nevertheless, it was easy to interpret them as positive on low power. Staining of the intraluminal portion of the benign papillomas varied depending on the degree of hyperplasia and sclerosis. In simple papillomas with prominent fibrovascular cores, CD10 and SMA showed an intact, continuous MEC layer. Positive nuclear staining was shown with p63 in MECs, although some cells that were thought to be MECs by H&E staining did not stain. Areas of florid hyperplasia within the papillomas were conspicuous for their seeming lack of staining in these areas with all 3 stains; however, closer examination revealed a faintly staining MEC layer at the base of the papillae in these cases.

All 06 cases of papillary carcinomas showed an absent basal MEC layer with no staining of the central portion of the lesions. These cases diagnosed as invasive carcinomas showed large, expansile, monomorphic, papillae in a variably desmoplastic or sclerotic stroma. The results of CD10, smooth muscle actin and p63, stains done were completely negative (Fig 3a, 3b, 3c, 3d) with good internal control samples in the form of nonneoplastic breast tissue. The results on H & E and IHC are summarized in Table 1.

Table 1: Categorization of Papillary lesions of breast on H&E and IHC

Diagnosis	H & E	IHC
Duct papillomas	05	05
Papillary epithelial hyperplasia	04	03
Papillary carcinomas	06	07
Total	15	15

Ductpapilloma

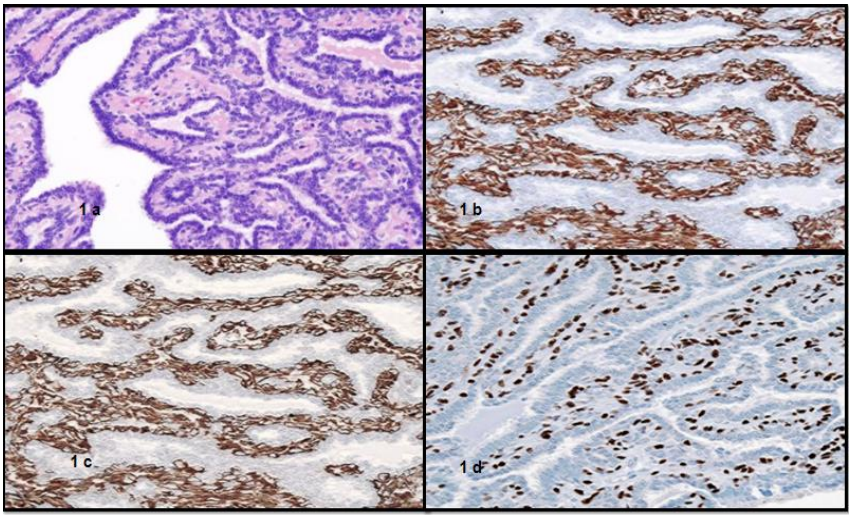

Fig 1: Intraductal papilloma. Typical intraductal papilloma showing a continuous myoepithelial cell layer (Fig 1a: H&E, 40X; 1b: CD 10, 40X; 1c: SMA, 40X; 1d: p63, 40X).

Papillary epithelial hyperplasia

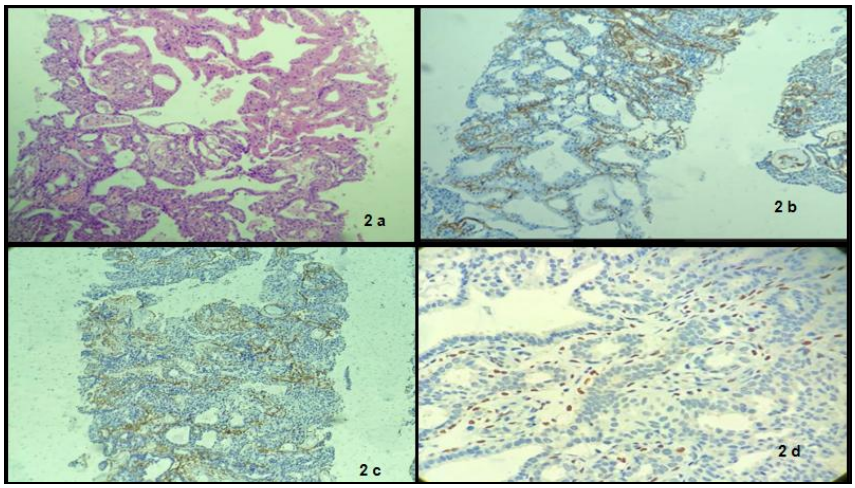

Fig 2: Papillary epithelial hyperplasia showing papillary architecture filling the spaces between papillae and an intact myoepithelial layer (Fig 2a: H&E, 40X; 2b: CD 10, 40X; 2c: SMA, 40X; 2d: p63, 40X).

Papillary carcinoma

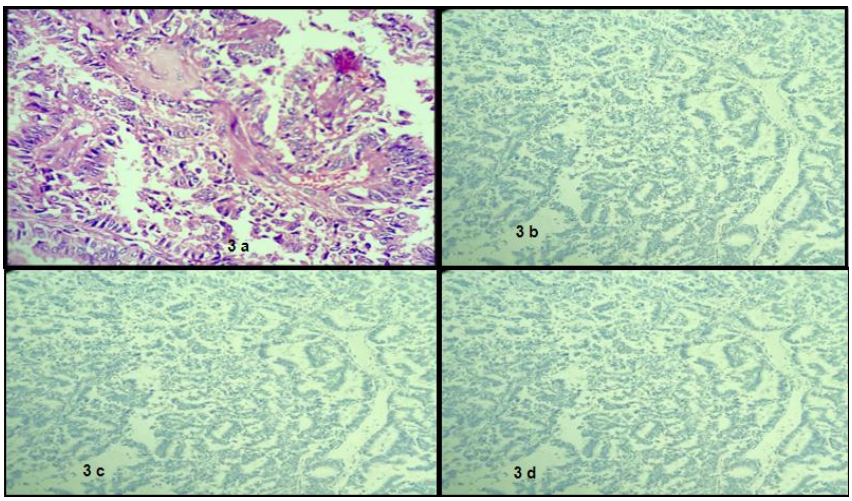

Fig 3: Papillary carcinoma showing tumor arrangement in the form of papillary clusters with central fibrovascular cores covered by malignant cells within a desmoplastic stroma. (Fig 3a: H&E, 40X; 3b: CD 10, 40X; 3c: SMA 40X; 3d: p63, 40X).

Discussion

Papillary carcinomas are relatively uncommon, accounting for only 1% to 2% of breast carcinomas comprising a diverse group of breast lesions that span the spectrum of hyperplastic and neoplastic processes.[1] A papillary breast lesion is defined as a proliferative lesion characterized architecturally by the presence of finger like projections composed of a stromal core overlaid by a layer of myoepithelial cells [1].

Intraductal Papilloma

An intraductal papilloma is a benign, proliferative breast lesion with a papillary architecture forming a mass that is confined within a duct. The number and distribution of papillomas enable them to be classified into solitary papilloma, mostly located in the large central and lactiferous ducts, and multiple papillomas, mostly located in the small and medium-sized peripheral ducts. Diffusely distributed microscopic papillary proliferations in the ducts as part of the spectrum of fibrocystic changes and Usual Ductal Hyperplasia are regarded as separate entities i.e., as papillary hyperplasia, rather than benign neoplasms.

Duct papillomas often present with nipple discharge, which is often bloodstained. They may also present as a mass, palpable or evident on imaging, and/or calcifications detected on mammogram. Imaging studies including ductography are often diagnostic for large, central papillomas. Ductoscopy with or without cytology examination brushings is performed in a few centers. Diagnosis on fine needle aspiration of papillomas has shown to have a significant false-positive rate. Today, diagnosis is commonly made by core biopsy, which provides only a sample of the lesion but has been shown to be highly accurate [6, 9]. Excision biopsy allows a thorough histologic assessment of the entire lesion.

Grossly, a papilloma is a friable nodule within a cyst formed by a dilated duct or as a solid nodule encapsulated by a sclerotic duct wall. Papillomas may extend into adjacent branches of the duct. On histology, simple papillomas have prominent sclerotic stromal cores, which is more extensive than the epithelial component. Calcifications may occur in the stroma. The papillary processes are covered by a layer of cuboidal or columnar luminal cells, with or without architectural complexity [10, 11]. Importantly, an MEC layer is interposed between the stroma and luminal cell layer. There is no cytological atypia and patchy apocrine metaplasia may commonly be encountered. Some papillomas show more focal MECs, which may aggregate into small nests. This feature raises the differential diagnosis of the papillary variant of adenomyoepithelioma [12]. In contrast to adenomyoepithelioma, in papillomas the MECs are increased only focally, lack nuclear atypia, and lack mitoses. Furthermore, the distribution of the MECs in papilloma is restricted to the basal zone of the epithelium, maintaining their normal spatial relationship to the luminal cells.

Invasive Papillary Carcinoma

Papillary carcinoma is rare, constituting 1-2% of breast cancers [12]. It can be non-invasive or invasive. The noninvasive form may extend throughout a ductal system (intraductal) or may be confined within a cystic structure (intracystic). The neoplastic epithelial cells are usually characteristic of low-grade DCIS. These cells are arranged in either solid, cribriform, micropapillary, or stratified spindle cell patterns [12]. Invasive carcinoma, when present, is usually detected at the periphery of the lesion. Invasive papillary carcinoma may have various growth patterns, either remaining a papillary pattern or, more commonly, having a nonspecific pattern. Underestimation of the degree of disease may occur by sampling error at percutaneous biopsy because the center of the lesion is often targeted, thereby missing the more aggressive elements that tend to be more peripherally located in these lesions.

This tumor usually occurs in older women and most commonly presents with a palpable mass. Bloody nipple discharge occurs in approximately 22-34% of patients [12]. Generally, patients with papillary carcinoma have a better prognosis, with less axillary nodal involvement than those with other forms of ductal carcinomas [13, 15] on mammography, papillary carcinoma is seen as a solitary round, oval, or lobulated circumscribed mass or as clusters of well-defined masses. Masses may have associated microcalcifications. Sonography reveals single or multiple circumscribed solid or complex mixed cystic and solid masses. These tumors tend to bleed centrally. Color Doppler sonography can show blood flow in the tumors and help differentiate them from blood clots. Papillary carcinoma is difficult to differentiate from benign papillomas using imaging features alone.

Pure invasive micropapillary carcinoma is rare, accounting for less than 2% of breast cancers, but a focal form of micropapillary growth has been reported in 3-6% of common types of invasive carcinomas. This lesion has distinctive histologic features characterized by the invasive micropapillary growth of tumor cells without a fibrovascular core. These tumor cells are polygonal to elongated with an eosinophilic to amphophilic cytoplasm surrounded by a clear space. This lesion occurs in the same age range as invasive ductal carcinoma of no particular type [16], with an extremely high incidence of regional lymph node involvement and a poor prognosis. Patients with this tumor may not benefit from a sentinel lymph node procedure

Importance of myoepithelial cells

The role of assessing myoepithelial cells in papillary lesions of the breast is twofold. The first is to identify the presence of myoepithelial cells that are interposed between the stromal fibrovascular cores and the overlying epithelial cells, and this is useful in the differentiation of papillary ductal carcinoma in situ and papilloma. The second role is to assess the presence or absence of a complete myoepithelial cell layer around the papillary lesion, particularly important in intracystic papillary carcinoma

The MEC layer is defined by the presence of immunoreactivity by CD10, SMA, p63 and newer MEC markers, which are are useful to distinguish benign from malignant and in situ from invasive lesions. The results showed consistent staining patterns with papillomas, papillary epithelial hyperplasias, and invasive papillary carcinomas [17]. Ductal papillomas showed positive staining in all CD10, SMA and p63. p63 showed irregularly spaced nuclear staining with occasional gaps. In simple papillomas with prominent fibrovascular cores, CD10 and SMA showed an intact, contiguous MEC layer. Cases of papillary epithelial hyperplasia showed positivity with all 3 stains. Cases with ADH showed variable staining which could

probably be indicative of the transformation into invasive papillary carcinoma [7]. Invasive papillary carcinoma showed large, expansive, papillary proliferations within a variably desmoplastic or sclerotic stroma [17]. CD10, SMA and p63 are virtually completely negative in these cases. The lack of a basal MEC layer suggests that these tumors might behave in an invasive manner [17].

The results obtained by us were in concordance with other studies conducted by Hill CB *et al* and Agoff *et al* which also showed that IHC staining for myoepithelial layer has proven to give a more accurate diagnosis in various papillary lesions of the breast.

Table 2: Comparison of sensitivity and specificity of IHC with other studies

Study	Sensitivity	Specificity
Present study	85.6%	100%
Hill CB *et al.* [2]	88%	98%
Agoff NS *et al.* [3]	90%	100%

Conclusion

It is difficult to differentiate benign from malignant lesions based on presence or absence of myoepithelial cell layer on H & E sections. It is prudent to employ a panel of IHC markers for definite identification of MEC layer, which would aid in more accurate treatment for the patient. p63, which is a nuclear marker is relatively more specific and sensitive when compared to CD10 and SMA which show cross reactivity with smooth muscle cells and myofibroblasts.

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