An immunohistochemical study for evaluation of CYTOKERATIN 5/6 AND S100 expression in intraductal proliferative breast lesions and ductal carcinoma in situ

Thesis

Submitted for Partial Fulfillment of Master Degree
In Pathology

By

Safinaz Talaat Anwar Ismail

M.B.,B.Ch. (Cairo University)

Supervisors

Prof. Dr. Fahima Mohamed Habib

Professor of Pathology
Faculty of Medicine
Cairo University

Prof. Dr. Samia Mohamed Gabal

Professor of Pathology
Faculty of Medicine
Cairo University

Prof. Dr. Tarek Nabil El-Bolkainy

Assistant Professor of Pathology
National Cancer Institute
Cairo University

Faculty of Medicine
Cairo University
2012

Acknowledgement

*First of all, thanks to "**ALLAH**", the most beneficent and merciful whom with only his help and generosity all works are done.*

Then, I would like to thank **Prof. Dr. Fahima Habib,** *Professor of Pathology, Faculty of Medicine, Cairo University for her general help and skillful support.*

I would like to express my profound gratitude to **Prof. Dr. Samia Gabal**, *Professor of Pathology, Faculty of Medicine, Cairo University for her supervision, patience and keen interest in conducting this work.*

My appreciation goes to **Assistant Prof. Dr. Tarek El- Bolkainy** *Assistant Professor of Pathology, National Cancer Institute, Cairo University for his kind cooperation and sincere help.*

Finally, I extend my deep thanks and love to my parents, brother and husband and all my family.

Also I appreciate all my staff members, colleagues and technicians of pathology department, Faculty of Medicine, Cairo University for their help and cooperation.

Safinaz Talaat Anwar Ismail

Abstract

Intraductal proliferative lesions of the breast have traditionally been divided into three categories: Usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS). However the term DCIS encompasses a highly heterogeneous group of lesions that differ with regard to their mode of presentation, histopathologic features, biological markers and risk for progression to invasive cancer. CK5/6 is of value in differentiating ductal proliferation of varying degrees especially in the differentiation between cancerous and non-cancerous changes. S-100 protein was shown to be present in myoepithelial and epithelial cells of the mammary gland. The aim of this study is to examine the immunohistochemical profiles for CK5/6 & S-100 in 40 Egyptian adult females' breast lesions with subsequent investigation of their potential diagnostic significance. The cases were subdivided into 25 cases of benign epithelial lesions including UDH, 5 cases of ADH, & 10 cases of DCIS. CK 5/6 positive expression was detected in 24 out of 25 benign epithelial lesions of the breast (96%), and was detected in four out of five atypical ductal hyperplasia (80%), and was detected in 2 out of 10 (20%) ductal carcinoma in situ. S100 positive expression in both epithelial and myoepithelial cells was detected in all benign epithelial lesions of the breast (100%), and four out of five atypical ductal hyperplasia (80%), and in one out of ten ductal carcinoma in situ (10%). It is noteworthy that S100 showed a higher level of significance than CK5/6, in distinction between malignant versus both atypical and benign lesions. The former also had a higher sensitivity, specificity and accuracy rates than CK5/6.So simultaneous detection of CK5/6 and S100 protein expression can help increase the diagnostic accuracy of breast diseases.

Key Words:

- Intraductal proliferative breast lesions.
- Usual Ductal Hyperplasia.
- Atypical Ductal Hyperplasia.
- Ductal Carcinoma in situ-CK5/6–S-100.

List of Content

List of Abbreviations

ADH	Atypical ductal hyprplasia
CK5/6	Cytokeratin 5/6.
DCIS	Ductal carcinoma in situ.
HMWK	High molecular weight cytokeratin
MGA	Microglandular Adenosis.
TDLU	Terminal duct lobular unit.
UDH	Usual ductal hyperplasia.
WHO	World health organization.

List of Tables

List of Figures

List of Graphs

Introduction

Although diseases of the breast number relativity fewer than those affecting many organs, the field of mammary pathology challenges the histopathologist as much as any discipline in surgical pathology. The patient's signs and symptoms and the other clinical details of the case do not give the pathologist much more than general guidance regarding the nature of the lesion. The macroscopic examination might suggest a diagnosis in certain cases, but many of the most troublesome lesions cannot be seen with the unaided eye. Thus it falls to the microscopist and to the microscopist alone to establish the diagnosis of the patient's disease ***(Frederick et al., 2009).***

Proliferative lesions of the breast represent a common and major pathologic diagnostic challenge. Atypical Ductal hyperplasia still remains a diagnostic dilemma with wide variation in both interobserver & intraobserver reproducibility among pathologists. The additioin of an Immunohistochemical stain (Cytokeratin High & low molecular weight cocktail) led to significant improvement in the concordance rate ***(Jain et al., 2011).***

There are several antibodies targeting cytoplasm (CK, S100, GFAP), membrane (E-Cadherin and HER-2), or nuclear (p53, p63, MIB-1), functional or structural antigens of epithelial and myoepithelial cells ***(Leong et al., 1999).***

A variety of studies have evaluated a number of prognostic tumor markers in the breast as a study carried by***(Helal et al,1997)*** To evaluate a number of prognostic tumor markers in infiltrating ductal carcinoma in a group of Egyptian women . However there is little information of immunohistochemical histologic profile of Egyptian adult females with proliferative lesions of the breast and its potential diagnostic significance. This urged us to evaluate the diagnostic significance of CK5/6 and S100 in categorization of proliferative breast lesions.

Aim of the Work

The current study aimed to evaluate the potential role of cytokeratin 5/6 expression in differentiating ductal proliferation of varying degrees especially in the differentiation between cancerous and non cancerous changes.

Comparison between the diagnostic significance of cytokeratin 5/6 and S100 immunoreactivity in the studied sample.

Testing the diagnostic validity of cytokeratin 5/6 and S100 expression in intraductal proliferations in the studied sample, regarding accuracy, sensitivity and specificity.

Structure of the Breast

The breast consists of 15–20 segments (lobes). Each segment is drained by a collecting duct. The segments are ill defined and cannot be identified by gross examination. Collecting ducts connect the nipple with lactiferous sinus. Segmental (lactiferous) and sub segmental (major) ducts connect lactiferous sinus with terminal duct-lobular units (TDLUs). Lobules are composed of terminal ducts and acini and their specialized supporting stroma. The terminal ducts are either extralobular or intralobular depending on their location relative to the specialized lobular stroma ***(Moinfar, 2007).***

The functional unit of the adult female breast from which the majority of both benign and malignant lesions arise is the terminal duct lobular unit ***(Silverberg et al., 2002).***

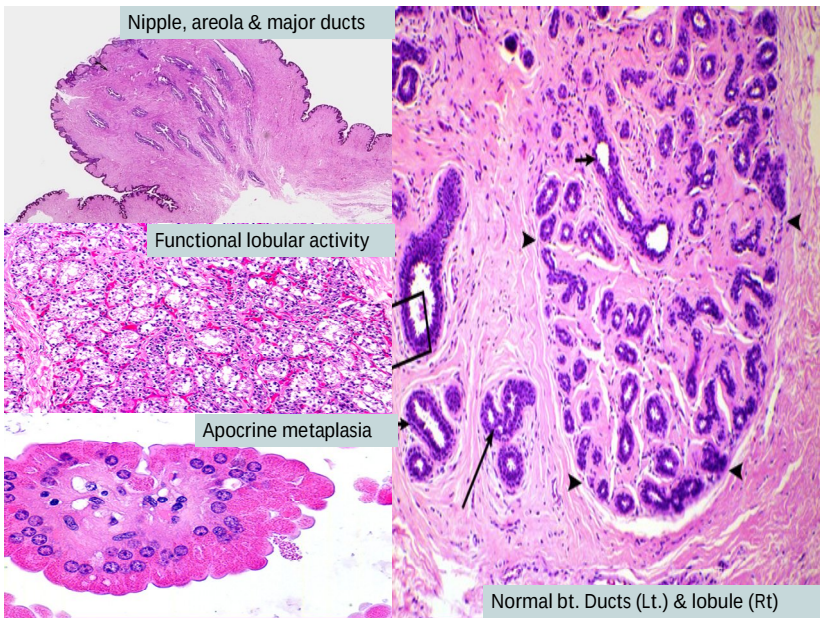

Fig (1):*Normal breast duct & lobule(**Mills et al., 2007**)*

The epithelium throughout the ductal-lobular system is bilayered, consisting of an inner (luminal) epithelial cell layer and an outer (basal) myoepithelial cell layer. The importance of this double cell layer cannot be overemphasized because it is one of the main guides used to distinguish benign from malignant lesions. These epithelial cells express a variety of cytokeratins, including cytokeratins 7, 8, 18,19 and 20. The outer (or myoepithelial) cell layer, although always present, is variably distinctive. Even when inconspicuous on hematoxylin- and eosin-stained sections, myoepithelial cells can readily be demonstrated using immunohistochemical stains for a variety of markers, including S-100 protein, actins, calponin, smooth muscle myosin, heavy chain, p63, and CD10, among others. However, these markers vary in both sensitivity and specificity for myoepithelium. Myoepithelial cells also express high molecular weight cytokeratins 5/6, 14, and 17. Work has documented the presence of a third cell type in normal breast tissue. These cells are dispersed individually and irregularly throughout the ductal-lobular system, express the basal cytokeratin CK5, and are thought to be progenitor cells capable of differentiating into both glandular epithelial cells and myoepithelial cells. However, the presence of such progenitor cells has not yet been universally accepted ***(Mills et al., 2007).***

The human breast epithelium is a branching ductal system composed of an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells that terminate in distally located terminal duct lobular units (TDLUs). While the luminal epithelial cell has received the most attention as the functionally active milk-producing cell and as the most likely target cell for carcinogenesis, attention on myoepithelial cells has begun to evolve with the recognition that these

cells play an active part in branching morphogenesis and tumor suppression ***(Gudjonsson et al., 2005).***

Myoepithelial markers

The presence of myoepithelial cells as the outer layer around ducts and lobules is a critical feature in distinguishing benign and non-invasive malignancies from invasive lesions, both in haematoxylin stained sections and immunohistochemically. However, expression of the wide range of proteins that are present in these cells can vary, even within normal breast tissue. The sensitivity and specificity of these proteins in the identification of myoepithelial cells vary between pathological conditions, and this is important for interpretation when these markers are used diagnostically. These markers fall into three main groups: smooth muscle related, cytokeratins (CKs) & others ***(Walker, 2007).***

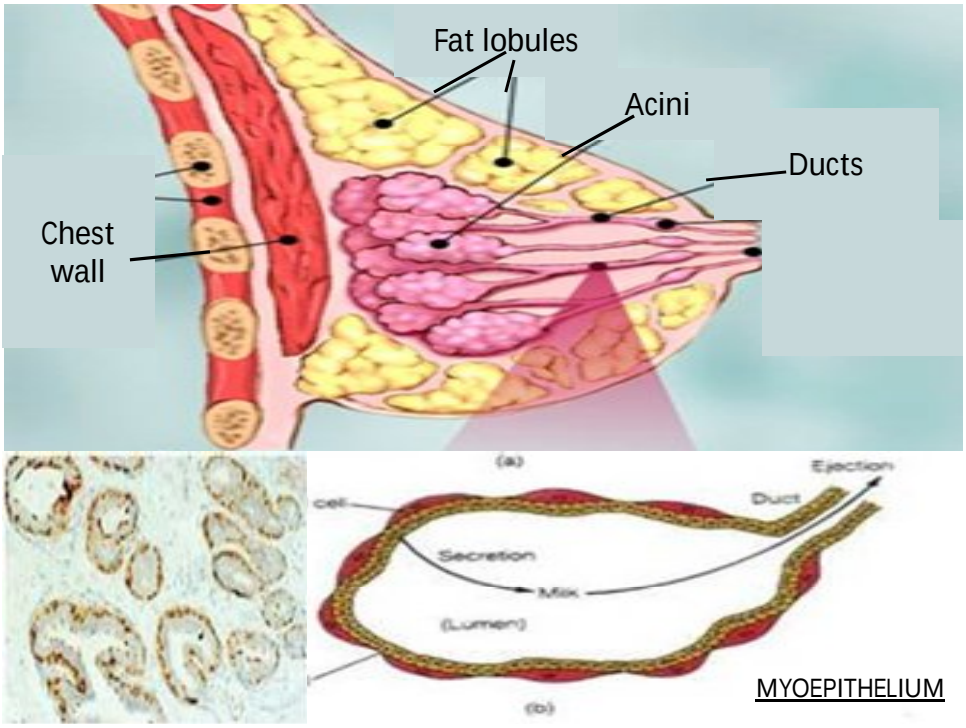

Fig (2):*Breast ducts &lobules with myoepithelial lining****(Mills et al., 2007)***