Case Report **IJCR (2020) 4:180**

International Journal of Case Reports
(ISSN:2572-8776)

HORMONE RECEPTOR EXPRESSION IN NON CANCER BREAST LESIONS

Arcot Rekha, Vimal Chander, Arihanth Ravichandran, Chitra S

Saveetha medical College, Saveetha Institute of Medical and Technical Sciences, Thandalam.

ABSTRACT

Background Estrogen receptors (ER), Progesterone receptors (PR) expression is seen in non cancer breast lesions like juvenile fibroadenomas and phyllodes tumour.

Materials and Methods This is a prospective study on a study population that comprised fibroadenomas and phyllodes tumour.

Results While a few studies indicate that ER/PR expression correlates with a more benign outcome,we did not see the same in our population.Ki 67 proliferation was greater in the stromal component of phyllodes tumours,compared to fibroadenomas.

Keywords: Fibroadenoma; Phyllodes tumour; Estrogen; Progesterone receptors; Ki67

***Correspondence to Author:**
Dr A.Rekha
Sukrithi,1/756,Sabari Nagar Extension, Mugallivakkam, Chennai 600125. Ph number: +91-9841241344

How to cite this article:
Arcot Rekha, Vimal Chander, Arihanth Ravichandran, Chitra S. Hormone Receptor Expression in Non Cancer Breast Lesions. International Journal of Case Reports, 2020; 4:180.

eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

Introduction

Development of breast neoplasia involves hormones such as estrogens and progesterone that regulate cell proliferation and apoptosis. The tissue-specific responsiveness to these hormones is partially regulated by the tissue expression of receptors that bind them. Ki67 is a cell proliferation marker, as it is present only during active phases of the cell cycle. Although expression of estrogen receptor α (ER), progesterone receptor (PR), and Ki67 serve as predictive and prognostic factors in breast cancer, little is known about their roles in non-carcinomatous breast tissue. Studies show that ER/PR expression correlates with benign behaviour while other studies don't prove this.

Aim and objectives of the project

To study the incidence of phyllodes tumour (PT) / juvenile fibroadenomas in our hospital

To document the clinical features and demographic profile of patients with PT and juvenile fibroadenomas

To peruse the pathology records and analyse the differentiation into benign and malignant PT.

To subject these slides to immunohistochemical (IHC) analysis and study their estrogen and progesterone receptor expression -ER/ PR

To analyse if there is a correlation between ER/PR expression and malignant potential of PT

To verify if there is a variation in ER/PR expression in juvenile fibroadenomas.

To ascertain the hormonal receptor profiles of the epithelial and stromal components of phyllodes tumors (PTs) and determine their relationship with stromal proliferation.

Materials and methods

Resected specimens of cases of breast lesions received in the Histopathology Laboratory, Department of Pathology, Saveetha Medical College Hospital over a period of 7 months from June 2018 to December 2018 were retrospectively retrieved from the files and analysed for the type of lesion/neoplasm and other prognostic features. The clinical features of these cases were retrieved from the medical records for the demographic profile. The slides and blocks of these cases were retrieved and then immunohistochemically analysed for Estrogen Receptor (ER) and Progesterone receptor (PR) expression and the results were statistically analysed.The inclusion criteria was juvenile fibroadenomas and phyllodes tumours(benign and malignant).we excluded all cases of carcinoma breast and equivocal and atypical lesions.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysisfor ER, PR and Ki67 were done in paraffintissue blocks using super sensitive HRP polymer system based on non-biotin polymeric technology. Sections of 4µ thickness were cut from the paraffin tissue blocks. They were transferred to gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with monoclonal antibody against Estrogen receptor (ER), Progesterone receptor (PR) and Ki67 proteins and then detected by the addition of secondary antibody conjugated with Horse Radish Peroxidase (HRP) polymer and Diaminobenzidine (DAB) substrate.

Annexure 1

IMMUNOHISTOCHEMISTRY PROCEDURE

4µ thick sections were cut from formalin fixed paraffin embedded tissue samples andtransferred to gelatin-chrome alum coated slides.The slides were incubated at 58^{o}C for overnight.The sections were deparaffinized in xylene for 15 minutes x 2 changes.The sections were dehydrated with absolute alcohol for 5 minutes x 2changes.The sections were washed in tap water for 10 minutes.The slides were then immersed in distilled water for 5 minutes.Heat induced antigen retrieval was done with microwave oven inappropriate temperature with appropriate buffer for 20 to 25 minutes.The slides were then cooled to room temperature and washed in running tapwater for 5 minutes.The slides were then rinsed in distilled water for 5 minutes.Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2changes. Apply peroxidase block over the sections for 10 minutes.Wash the slides in phosphate buffer for 5 minutes x 2 changes.Cover the sections with power block for 15 minutes.The sections were drained (without washing) and appropriate

primaryantibody was applied over the sections and incubated for 45 minutes.The slides were washed in phosphate buffer for 5 minutes x 2 changes.The slides were covered with Super Enhancer for 30 minutes.The slides were washed in phosphate buffer for 5 minutes x 2 changes.The slides were covered with SS Label for 30 minutes.Wash in phosphate buffer for 5 minutes x 2 changes.DAB substrate was prepared by diluting 1 drop of DAB chromogento 1ml of DAB buffer.DAB substrate solution was applied on the sections for 8 minutes.Wash with phosphate buffer solution for 5 minutes x 2 changes.The slides are washed well in running tap water for 5 minutes.The sections were counterstained with Hematoxylin stain for 2 seconds (1dip).The slides are washed in running tap water for 3 minutes.The slides are air dried, cleared with xylene and mounted with DPX.

INTERPRETATION AND SCORING SYSTEM

The immunohistochemically stained slides were analysed for the presence of reaction, cellular localization (cytoplasm and cytoplasmic membrane or nuclear staining) and the percentage of cells stained and scores as follows.Allred scoring system(table 1) for Estrogen receptor (ER) and Progesterone receptor (PR) expression:

Allred scoring system-Table 1

ALLRED SCORING SYSTEM		
	Score	Percentage of tumor cells showing nuclear positivity
Proportion Score	1	<1 %
	2	1 to 10 %
	3	11 to 33%
	4	34 to 66 %
	5	67 to 100 %
	Score	Intensity of Staining
Intensity Score	1	Weak
	2	Intermediate
	3	Strong
TOTAL SCORE = Proportion Score + Intensity score		
Minimum score = 1 + 1 = 2 Maximum score = 5 + 3 = 8		

For Ki67, the percentage of cells showing nuclear positivity and the intensity of staining (Weak, Intermediate or Strong) is noted.

Ki67 SCORING SYSTEM	
Percentage of tumor cells showing nuclear positivity	Interpretation
< 10%	Low
10 to 20%	Borderline
> 20%	High

RESULT

We found a total of 18 women matching the inclusion criteria and they formed our study population. We had 44.4% presenting with cellular fibroadenoma, 38.9% presenting with benign phyllodes tumor and 16.7% presenting with malignant phyllodes tumor(Fig 2) based on the H&E staining of the slides. There was maximal clustering of cases in the 20-30 years of life (fig1 1)

and there were very few cases of malignant phyllodes in our study population. [1,2]

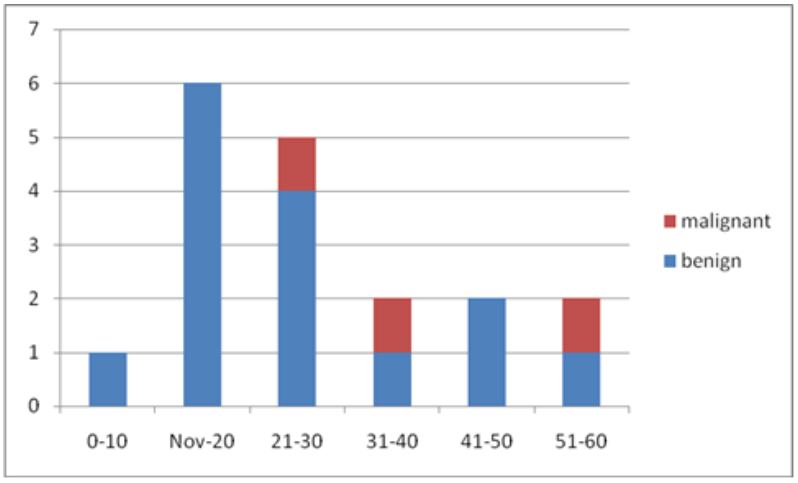

Fig 1 shows the distribution of the tumours across the age intervals

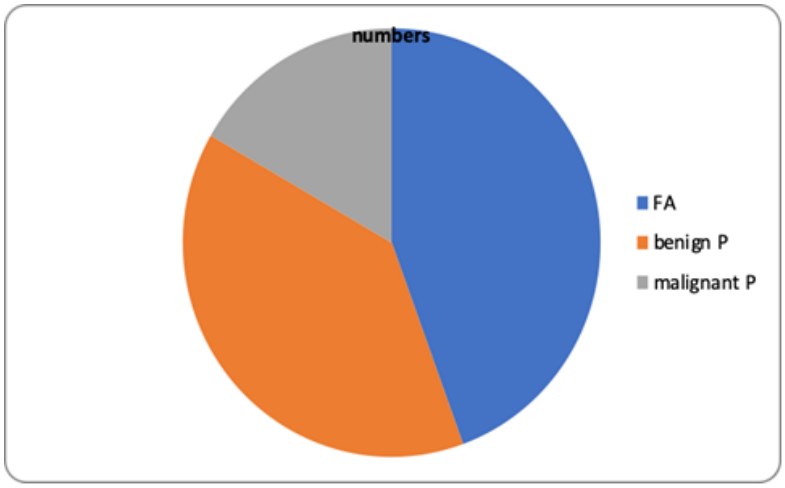

Fig 2 The distribution of cellular fibroadenoma and phyllodes

The mean age of the patients with fibroadenoma was 18.25(range 14-21) for benign phyllodes it was 37 years (range 29-51) and for malignant phyllodes 39.7 years (30-55).

When we analysed the ER and PR staining in fibroadenoma all epithelial elements stained positive and all mesenchymal elements stained negative. 87.5% of the epithelial elements stained positive and 37.5% of mesenchymal elements stained positive for Ki-67 (Fig 3) These two results, together with the young age of patients carrying fibroadenomas with highly ER positive cells, may further indicate a hormone-receptor mechanism involved in regulating the growth of fibroadenomas.

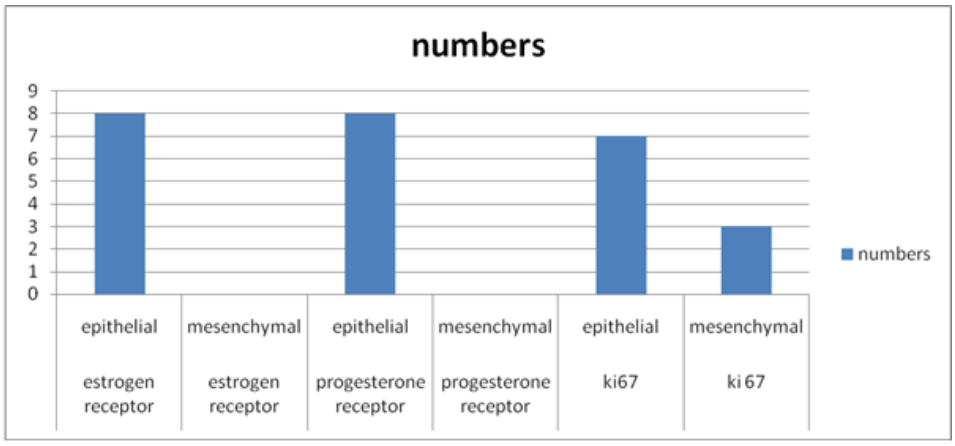

Fig 3 shows ER/PR /Ki67 expression in fibroadenomas

Amongst benign phyllodes tumor, ER and PR stained positive for epithelial elements in 71.4% of the patients and all were negative for the mesenchymal elements (100%). Ki-67 was positive for 71.4% of the epithelial elements and 100% positive for the mesenchymal elements (Fig 4).

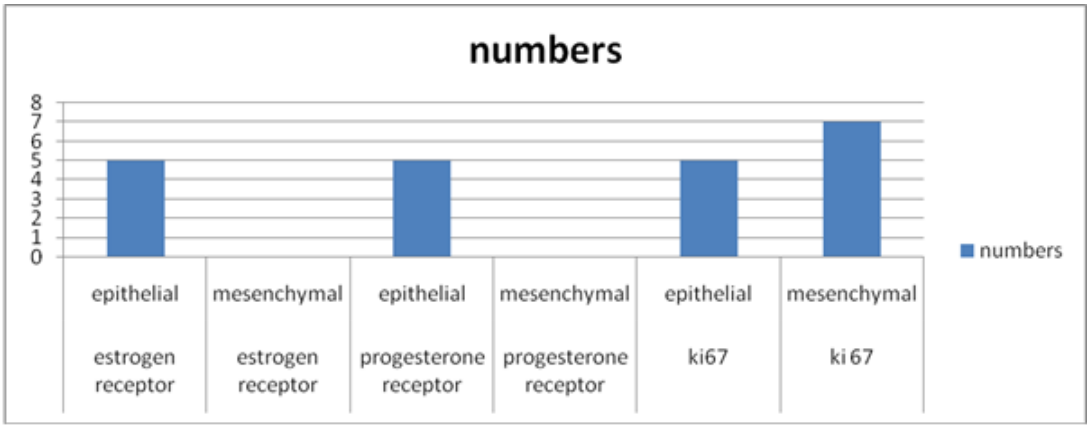

Fig 4 shows ER/PR/Ki67 expression in benign phyllodes

We had only three patients with malignant phyllodes tumor. 100% of these tumors were ER/PR positive for the epithelial elements and negative for the mesenchymal elements. Ki-67 was 33.3% positive for all the epithelial elements and 100% positive for the mesenchymal elements in malignant phyllodes. When we looked at the mesenchymal positivity for Ki-66 across all patients -72.2% were positive (Fig 5).

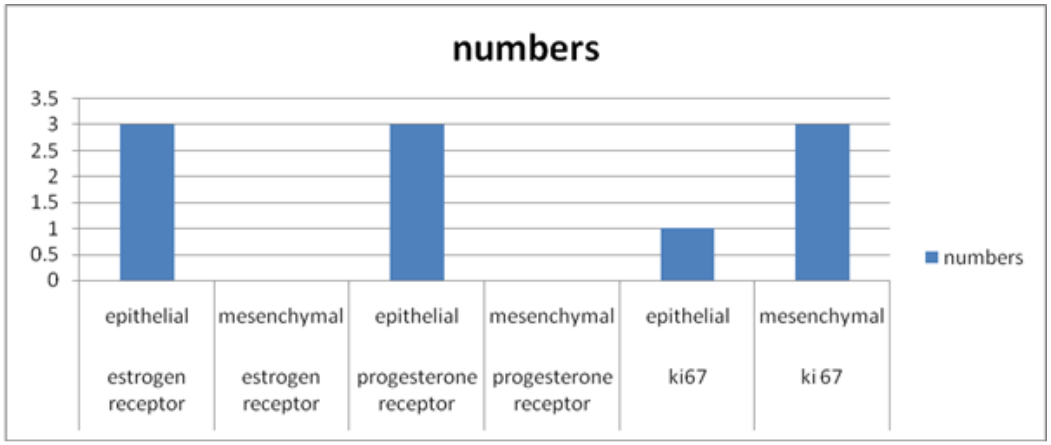

Fig 5 shows ER/PR/Ki 67 expression in Malignant phyllodes

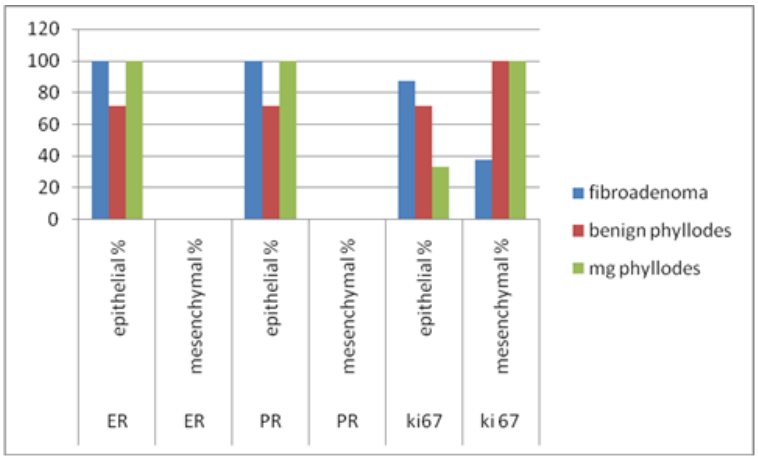

Fig 6 shows ER/PR/Ki67 expression (as a percentage) in the epithelial and mesenchymal components of fibroadenoma,benign and malignant phyllodes

In our small sample study (fig 6) we found that epithelial components of fibroadenoma,benign phyllodes(BP) and malignant phyllodes(MP) was positive 100 %,71.4%,100% respectively for ER. We also found that mesenchymal components of fibroadenoma,benign phyllodes(BP) and malignant phyllodes(MP) was always negative for ER. We found that epithelial components of fibroadenoma,benign phyllodes(BP) and malignant phyllodes(MP) was positive 100%, 71.4%, 100% respectively for PR. We also found that mesenchyamal components of fibroadenoma, benign phyllodes (BP) and malignant phyllodes (MP) was always negative for PR. Ki 67 expression in the epithelial component of fibroadenomas,BP and MP were87.5%,71.4% and 33.3% respectively. . Ki 67 expression in the mesenchymal component of fibroadenomas,BP and MP were 37.5%, 100% and 100% respectively.The progesterone receptors in malignat phyllodes had the highest Allred score followed by the PR in cellular fibroadenoma (Fig 7)

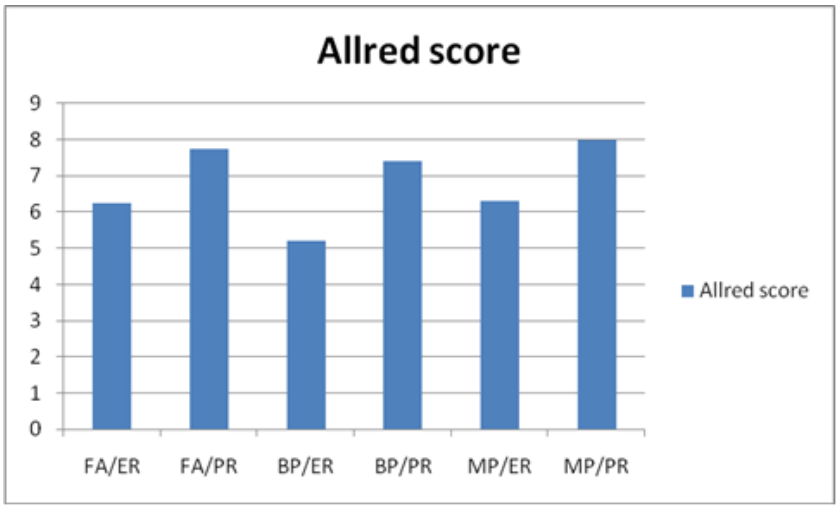

Fig 7 shows the Allred Score in FA,benign and malignant phyllodes

Discussion

Breast neoplasia is regulated by estrogen and progesterone hormones that regulate cell proliferation and apoptosis. The tissue specific responsiveness to these hormones is partially regulated by tissue expression of receptors that bind to them. Ki 67 is present during the active phase of the cell cycle and is a proliferation index. ER, PR and Ki 67 are regularly done in cases of carcinoma breast as it has prognostic implications and targeted therapy is now standard of care. Tamoxifen and SERM use however is not routine in management of phyllodes tumour. [3,4]

Cellular fibroadenoma mimic phyllodes tumour. The histogenesis of fibroadenoma and phyllodes

tumour seems similar. Mammary phyllodes tumor (PT) is an uncommon stromal-epithelial neoplasm with a reported incidence of 0.3% to 0.5% of female breast tumors.The median and mean ages of patients are 45 years, and the average tumor size is 4 to 5 cm. Rarely these lesions can occur in young or elderly women or in men. Pathologically, PTs are divided into benign and malignant categories based on assessment of a combination of histologic features, namely mitotic count, cellularity and nuclear pleomorphism of the stromal cells, stromal overgrowth, and circumscription of the tumor border. The propensity to recur makes proper and adequate treatment imperative, even in benign cases. Currently the mainstay of treatment of mammary PT remains surgical, and this also applies to recurrences and metastases. Several studies have documented the presence of ER, PR receptors in both the epithelial and the mesenchymal elements of benign and malignant phyllodes. The results from several studies are variable and inconsistent.

Studies show that hormone receptor expression is more in the epithelial components and absent or less in the stroma. Other studies have shown that receptor expression is more in benign phyllodes tumour and hence can be used to prognosticate patients and help decide pre-operatively if the tumour requires a mastectomy rather than a wide local excision. However other studies showed that there is no correlation between receptor expression and tumour grade. Ki67. a proliferative marker was increased in all cases of increased cellularity. [5] There seems to be a relationship between ER and PR expression and the degree of malignancy of PTs. For ER, the expression was high in benign PTs but low in borderline and malignant PTs. For PR, the expression was high in both benign and borderline PTs but low in malignant PTs. This is particularly interesting, as the expression of ER was in the epithelium, and the mitotic count was determined from the stromal cells. This correlation raises the possibility that the epithelium may have a significant role in triggering the stromal proliferation, and this mechanism may be reflected partly in the histomorphologic features of subepithelial stromal condensation. This type of stromal-epithelial interaction and cell signalling that may lead to epigenetic changes in the stroma has been observed in other tumors, such as prostatic neoplasms. [6,7] Third, as PTs progress from benign to borderline to malignant, the level of ER expression decreases before the level of PR expression does, so in borderline PTs, there is a low level of ER expression but PR expression remains high. It is only when the PTs reach the malignant stage that both ER and PR expression decrease to a low level.

Conclusions

We found very few patients with juvenile fibroadenomas.Younger patients were found to have benign lesions and only in the 4th decade did the occurrence of malignant lesions start.Malignant phyllodes is very uncommon in our study group. ER/PR expression was high in the epithelial component of the fibroadenoma and the benign phyllodes group but not often seen in the mesenchymal component. Ki 67 expression was higher in epithelial component in fibroadenomas compared to the mesenchymal component.In benign and in malignant phyllodes,Ki 67 expression was more in the mesenchymal elements compared to the epithelial,indicating that the stromal component is more active in phyllodes.We were unable to prove that greater ER/PR receptors were present in benign phyllodes compared to malignant phyllodes.The Allred score was higher for PR receptors than ER in fibroadenomas,benign and malignant phyllodes.

References

[1] Kim YH, Kim GE, Lee JS, Lee JH, Nam JH, Choi C, Park MH, Yoon JH. Hormone receptors expression in phyllodes tumors of the breast. Anal Quant Cytol Histol. 2012 Feb;34(1):41-8.

[2] Deepti Gupta, Veena Gupta, Nisha Marwah, Meenu Gill, Sumiti Gupta, Gopal Gupta, Promil Jain, and Rajeev Sen Correlation of Hormone Receptor Expression with Histologic Parameters in Benign and Malignant Breast Tumors. Iran J Pathol. 2015 Winter; 10(1): 23–34.

[3] Giani C, D'Amore E, Delarue JC, Mouriesse H, May-Levin F, Sancho-Garnier H, Breccia M, Contesso G. Estrogen and progesterone

receptors in benign breast tumors and lesions: relationship with histological and cytological features. Int J Cancer. 1986 Jan 15;37(1):7-10.

[4] Mudduwa L, Liyanage T. Immunohistochemical assessment of hormone receptor status of breast carcinoma: interobserver variation of the quick score.Indian J Med Sci. 2009 Jan;63(1): 21-7.

[5] Kucuk U, Bayol U, Pala EE, Cumurcu S. Importance of P53, Ki-67 expression in the differential diagnosis of benign/malignant phyllodes tumors of the breast. Indian J Pathol Microbiol. 2013 Apr-Jun;56(2):129-34. doi: 10.4103/0377-4929.118690.

[6] Tse GM, Lee CS, Kung FY, Scolyer RA, Law BK, Lau TS, Putti TC. Hormonal receptors expression in epithelial cells of mammary phyllodes tumors correlates with pathologic grade of the tumor: a multicenter study of 143 cases. Am J Clin Pathol. 2002 Oct;118(4):522-6.

[7] Shoker BS, Jarvis C, Clarke RB, et al. Abnormal regulation of the oestrogen receptor in benign breast lesions. J Clin Pathol. 2000;53:778-783.