

Clinicopathological profiles and ki67 expression of luminal B breast cancer in Hospital Pakar Universiti Sains Malaysia

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ABSTRACT

Objective

Breast cancer has a growing incidence among Malaysian women. Its molecular classification, based on immunohistochemical (IHC) staining, has been widely used as a guide for therapeutic decision making. Luminal B is an aggressive molecular subtype with poor outcomes. This study aimed to determine the prevalence, prognostic parameters, and outcomes of luminal B, distinguished from luminal A subtype by a nuclear marker, Ki67, in the female breast cancer population in Hospital Pakar Universiti Sains Malaysia (HPUSM).

Materials and methods

A retrospective study included 165 female patients with luminal breast cancer (ER/PR+, HER2- and HER2+) treated at HPUSM from years 2009-2018. Quantitative analysis of stained luminal A slides via Ki67 antigen was performed. Positive slides (cut-off level >20%) were termed luminal B HER2- subtype, and the clinicopathological features recorded. Cross-tabulated statistics of recorded data were performed for the luminal B subtypes. Statistical significance was measured using Chi Square test, Fisher's exact test and Mann-Whitney U t-test, where appropriate. Statistical analysis was done using SPSS version 26.

Results and Discussion

85 luminal B cases were reported. Among the stained luminal A cases, 19% were positive, and termed luminal B HER2-. Luminal B cancers were prevalent in premenopausal women (52%), with parity >3 (62%); associated with high grade (46% grade 2) and stage (44% stage 4). 34% patients had recurrence within 5 years of diagnosis. 70% had lymph node involvement and 45% had lympho-vascular invasion. Lung and bone were the most common sites of metastasis, while nodular metastasis was strongly linked to recurrence (34.5% patients).

Conclusion

Luminal B was shown to have high grade, stage, metastasis, and recurrence, while differentiating from luminal A via the ki67 index. Owing to its disease course, early diagnosis and management are essential in improving patient outcomes.

Keywords

breast cancer, ki67 antigen, estrogen receptor, progesterone receptor, erbB-2 Receptor

INTRODUCTION

Breast cancer remains the most common malignancy affecting women worldwide¹, and its incidence continues to rise steadily among women in Malaysia². Advances in microarray-based gene expression profiling have revealed that the treatment response in breast cancer is largely influenced by its intrinsic molecular characteristics, rather than by traditional anatomical prognostic indicators³. This understanding has introduced a new paradigm in breast cancer classification and management, enhancing both the precision of prognostic assessment and the tailoring of therapeutic strategies³.

According to the St. Gallen International Consensus, breast cancer can be categorized into molecular subtypes based on immunohistochemical (IHC) expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). These include luminal A (ER/PR positive, HER2 negative), luminal B (ER/PR positive, HER2 negative or positive), HER2-enriched (ER/PR negative, HER2 positive), and basal-like or triple-negative (ER/PR/HER2 negative) types⁴.

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The luminal B group accounts for approximately one-third of breast cancers^{4,5}. Tumors of this subtype tend to have lower ER and PR expression, higher histologic grade, and a more aggressive clinical course compared to luminal A cancers^{6,7}. They are also associated with a higher rate of recurrence and shorter disease-free survival^{5,6}.

As described by the St. Gallen panel, Ki-67, a nuclear proliferation marker, helps distinguish luminal B from luminal A and other molecular classes. For HER2-negative luminal B cancers, a Ki-67 index above 20% is typically used as a cut-off, though some studies suggest extending the threshold to 20–29%, which remains an area of ongoing debate⁸. This classification framework not only aids therapeutic decision-making but also holds prognostic significance by correlating with disease presentation, recurrence trends, and survival outcomes⁴.

This study aims to assess the prevalence and clinicopathological characteristics, such as lymph node involvement and recurrence, of luminal B breast cancers among female patients at Hospital Pakar Universiti Sains Malaysia (HPUSM). Differentiation from the luminal A subtype was achieved using IHC profiling and Ki-67 immunostaining. Demonstrating that tumor behavior can be characterized without advanced molecular diagnostics emphasises the practical value of IHC and Ki-67 in reflecting intrinsic tumor biology. Early identification of luminal B disease patterns may therefore contribute to improved patient management and outcomes.

MATERIALS AND METHODS

The study was a retrospective study. Selected population included 165 female patients diagnosed with invasive breast carcinoma; with prior IHC staining showing ER/PR+, HER2– and HER2+ (luminal A and B) recruited from HPUSM, from years 2009-2018. The clinical information including age, ethnicity, parity, menopausal status, tumor stage and grade, lymph node status, metastasis, and 5-year recurrence, was retrieved from medical records. Inclusion criteria were female patients undergoing or who underwent treatment at HPUSM while the excluded population were male patients, female patients with incomplete records, missing laboratory samples and non-luminal breast cancers.

ER/PR status

The ER/PR status was determined by IHC staining, with >1% staining considered a positive result. Tumors were considered HER2+ if the score was 3+ by IHC staining. In case the score was 2+, Fluorescence in situ hybridization (FISH) or Dual-color dual-hapten in situ hybridization (DDISH) was checked for HER2 gene amplification and subsequently considered HER2+. This information was obtained from case records. Cases with HER2 score 2+, where FISH/DDISH was not done, were excluded. Nodal positivity was defined as the presence of any tumor cells in the lymph nodes.

Ki67 index

All available luminal A samples (ER/PR+, HER2–) were stained with the Ki67 stain. The staining was performed on formalin fixed paraffin embedded tissue (FFPE) with MIB1 / DAKO as the clone marker for Ki67; dilution ratio was 1:200 and antigenic retrieval was done using a citrate buffer, pH 9 (water bath, 20 minutes). Positive and negative tissue controls were included for the Ki67 IHC stain; prepared from tonsil tissue (Fig 1), with a TBS buffer used for the negative control slides^{17,18}

Ki67 score was expressed as a percentage of the number of IHC stained nuclei among the total number of nuclei of tumor cells regardless of the immunostaining intensity. The counting was performed in three randomly selected fields of the tumor tissue section at x40 magnification. When the staining was homogeneous, two peripheral and one central field were used for counting¹⁹.

Ki67 score range was from 0 to 100% and the positive cut-off level used was $\geq 20\%$ ⁸ (Fig 2 and 3).

Expression of the marker was evaluated by our breast pathologist at HPUSM (competency updated with regular competency assessment done in the accredited laboratory by standard Malaysia MS ISO 15189).

Data analysis

All categorical data is presented in frequency and percentage, while the numerical data is presented in mean and standard deviation. Comparisons between two groups, was made using the Chi Square test, Fisher's exact test and the Mann-Whitney U t-test, as appropriate. P value of less than 0.05 was considered statistically significant. Statistical analysis was performed using version 26 of the SPSS software (SPSS Inc, Chicago, IL).

RESULTS

Clinical characteristics

Among a total of 165 luminal cancer cases, 85 luminal B cases and 80 luminal A cases were reported from years 2009 to 2018. The greatest number of breast cancer cases were in 2017, where the prevalence of luminal B was 15%. The luminal A cases (ER/PR+, HER2-) were stained with Ki67, out of which 19% were positive, and termed luminal B HER2-. A cut-off level of 20% was used.

The median age of the patients was 48 and predominant ethnicity Malay. 52% of patients were pre-menopausal with a median parity of 3. The left breast was more commonly affected and median tumor size 3.2 cm. 70% of patients had lymph node involvement, with most patients having more than 2 lymph nodes involved. 46% of tumors were grade 2 and 44% were stage 4.

45% patients had LVI and 34% had a recurrence of cancer within five years.

Lung and bone were the most common sites of metastasis. 7 deaths were reported. (Table 1).

Metastasis

There was a significant association between tumor size and metastasis ($p < 0.001$). Tumor size was higher among patients with metastasis. Metastasis patients had 62.9% of 2 or more lymph node involvement compared to patients with no metastasis ($p = 0.003$). Patients with cancer grade 2 and stage 4 with metastasis were higher (52% and 60%) compared to other grades ($p = 0.005$, $p < 0.001$), along with 64% of patients with LVI and metastasis ($p = 0.025$). Other associations were not significant. (Table 2).

Recurrence

There was a significant association between nodular metastasis and recurrence of cancer ($p = 0.033$) where 34.5% of patients with metastasis had recurrence.

There were no significant associations between other factors and recurrence. (Table 3).

Mortality

There was a significant association between other metastases, including brain, and death ($p = 0.023$), where 43% of patients with other metastases died. All patients that died had LVI ($p = 0.048$). Other associations were not significant.

DISCUSSION

The rising incidence of breast cancer among Malaysian women continues to be a pressing public health concern. According to the Malaysian National Cancer Registry, approximately one in thirty Malay women is affected by this disease¹. The present study assessed the clinicopathological characteristics of luminal B breast cancer, which represented 15% of all cases, aligning with international data; 19% in China, 17% in the United States, 16% in Tunisia, and 16% in Saudi Arabia²⁰. Variables examined included age, parity, menopausal status, tumor grade and stage, lymph node involvement, metastasis, and recurrence. In comparison, a Turkish study reported that 20% of breast cancer patients were below 40 years of age, with nearly half being premenopausal²¹.

Younger, premenopausal women with higher parity have been shown to have worse prognoses in luminal B (HER2-) cancers^{21,22}. The estrogen receptor (ER) plays a role in mediating pregnancy-related hormonal changes, stimulating mammary epithelial proliferation²³. Additionally, alternative signaling pathways, particularly those involving epidermal growth factor receptors (EGFR), may explain the relationship between parity and adverse outcomes in luminal B tumors²². In the current cohort, the mean patient age was under 50 years, with 52% being premenopausal and parity of three, findings consistent with earlier studies. Although a slight rise in parity ($n=4$) was noted among HER2- cases, this did not reach statistical significance in relation to patient outcomes.

Luminal B breast cancers are known for their more aggressive biological behavior, typically presenting with larger tumors, advanced nodal disease, and higher stages compared to other molecular subtypes²⁰. In this study, the mean tumor size was 3.2 cm, and 70% of patients had nodal involvement, with most having two or more affected nodes. Tumor grade 2 accounted for 46% of cases, while stage 4 disease was present in 44%. Larger tumor dimensions, higher grade, and advanced stage were all significantly associated with increased risk of metastasis.

Previous research has reported a higher frequency of local recurrence and distant spread (up to 66%), often involving the bone, within five years of diagnosis of luminal B cancer^{7,24,25}. Similar findings were observed in this study, where 73% of patients developed metastases,

primarily to the bone and lung, and 34% experienced recurrence within five years. Nodal metastasis was significantly linked to recurrence, with 35% of node-positive patients developing recurrence. Involvement of the brain was associated with particularly poor outcomes, with 43% mortality within ten years.

The nuclear marker Ki-67 plays an important role in the prognosis of luminal breast cancers, strongly influencing disease-free survival, with routine use in clinical work^{26,27}. Accurate assessment of Ki-67 relies on IHC staining, which allows for localization and semiquantitative evaluation of protein expression within tissue sections. The reliability and reproducibility of IHC have been demonstrated across diverse tissue types, including ovarian stromal tumors²⁸ and fetal thymus²⁹, supporting its application in breast cancer. Ki-67 expression has been associated with higher histopathological grade, lymphovascular invasion (LVI), and lymph node involvement³⁰.

Moreover, Ki-67 functions as an independent prognostic indicator, complementing other clinicopathological parameters and predicting response to neoadjuvant chemotherapy. A Japanese study found that the mean Ki-67 index in patients achieving pathological complete response (pCR) was 51, compared with 27 in those without pCR²⁶. A higher Ki-67 level correlated with better chemotherapy response and improved overall survival, whereas patients with lower indices (<20%), characteristic of luminal A tumors, showed superior disease-free survival with endocrine therapy²⁶. Collectively, these findings reinforce the role of Ki-67 not only as a prognostic marker but also as a predictive indicator for therapeutic response, particularly in luminal B tumors with aggressive clinical behavior. Given its ability to reflect tumor proliferative activity, Ki-67 is a key parameter in distinguishing luminal B from luminal A tumors in HER2- cases, which has important implications for patient management^{13,14}.

The HER2 positive status of tumors remains an independent predictor of survival and metastasis, alongside Ki-67 index⁷. However, a notable variance was found in comparative studies, which showed a higher predilection for bone metastasis in HER2- compared to HER2+ subtypes⁷. Consequently, the

St. Gallen Consensus reclassified a subset of tumors previously classified luminal A (HER2-), as luminal B when a high Ki-67 index was present; owing to the more aggressive clinical behavior and poorer clinical outcomes of luminal B tumors^{13,14}. In this study, 19% of luminal A (HER2-) tumors were reclassified as luminal B based on amplified Ki-67 expression. These findings highlight the importance of combined evaluation of HER2 status and Ki-67 index for accurate molecular classification, prognosis, and therapeutic planning.

LVI is another negative prognostic indicator in luminal B cancers, serving as an early marker of metastatic potential and being closely associated with poor survival outcomes^{15,16}. In this cohort, 45% of patients exhibited LVI, and 64% of those developed metastases. Furthermore, LVI was present in all patients who died within ten years of diagnosis.

In conclusion, luminal B breast cancer represents a biologically aggressive molecular subtype characterized by high proliferation rates, frequent metastasis, and recurrence. This study highlights the distinguishing features of luminal B from luminal A tumors through HER2 status and Ki-67 labelling index. Given the limited literature exploring the reclassified subset of luminal A patients as luminal B, these findings emphasize the value of early detection and the prognostic utility of Ki-67.

Among the study's limitations were incomplete datasets and loss to follow-up in some patients. Additionally, the manual assessment of Ki-67 may introduce observer variability compared with automated image-based analysis used in other studies³¹. The adoption of the 20% cut-off for Ki-67 also limited comparison with older studies employing different thresholds.

Nevertheless, the nine-year study duration, adequate sample size, and in-house IHC processing provide valuable insights into luminal B breast cancer patterns at HPUSM. These results contribute to the understanding of its molecular heterogeneity and reinforce the need for routine Ki-67 evaluation in clinical practice. Future research should aim to refine therapeutic targets and optimize treatment strategies, ultimately improving patient survival and disease management outcomes.

Table 1. Patient and Clinical Characteristics of luminal B breast cancer (n=85)

Variable		n	(%)	Median	(IQR)
Age				48	14
Ethnicity	Malay	79	92.9		
	Chinese	6	7.1		
Menopausal	pre	44	51.8		
	post	41	48.2		
Parity				3.0	2.00
HER2	pos	70	82.4		
	neg	15	17.6		
Breast Affected	left	41	48.2		
	right	39	45.9		
	Bilateral	5	5.9		
Tumour Size (cm)				3.20	1.4
Lymph Nodes Involvement	0	26	30.6		
	<2	14	16.5		
	2	14	16.5		
	>2	31	36.5		
Cancer Grade	1	10	11.8		
	2	39	45.9		
	3	36	42.4		
One or more Metastasis	No	23	27.1		
	Yes	62	72.9		
Nodular Metastasis	No	60	70.6		
	Yes	25	29.4		
Lung Metastasis	No	53	62.4		
	Yes	32	37.6		
Bone Metastasis	No	54	63.5		
	Yes	31	36.5		
Liver Metastasis	No	65	76.5		
	Yes	20	23.5		
Other Metastasis	No	73	85.9		
	Yes	12	14.1		
Cancer Stage	1	9	10.6		

Variable		n	(%)	Median	(IQR)
	2	30	35.3		
	3	9	10.6		
	4	37	43.5		
LVI	No	30	35.3		
	Yes	38	44.7		
Recurrence	No	39	45.9		
	Yes	29	34.1		
Mortality	No	78	91.8		
	Yes	7	8.2		

Table 2. Factors associated with Metastasis in the luminal B population (n=85)

Variable		No Metastasis		Metastasis		
		N	(%)	N	(%)	p-value*
Age	(Median, IQR)	46.0	8.0	49.0	16.0	0.216^
Ethnicity	Malay	20	87.0%	59	95.2%	0.337
	Chinese	3	13.0%	3	4.8%	
Menopausal	pre	14	60.9%	30	48.4%	0.306
	post	9	39.1%	32	51.6%	
Parity	(Median, IQR)	2.00	3.00	3.00	2.00	0.065^
HER2	pos	18	78.3%	52	83.9%	0.547
	neg	5	21.7%	10	16.1%	
	Ki67 (n=15)	0.24	0.04	0.23	0.13	0.594^
Breast Affected	left	15	65.2%	26	41.9%	N/A
	right	8	34.8%	31	50.0%	
	B/I	0	0.0%	5	8.1%	
Tumor Size (cm)	(Median, IQR)	2.20	0.90	3.50	1.20	<0.001^
Lymph Nodes Involvement	<2	17	73.9%	23	37.1%	0.003
	2 and more	6	26.1%	39	62.9%	
Cancer Grade	1	9	39.1%	1	1.6%	0.005^

Variable		No Metastasis		Metastasis		p-value*
		N	(%)	N	(%)	
Cancer Stage	2	7	30.4%	32	51.6%	<0.001^
	3	7	30.4%	29	46.8%	
	1	6	26.1%	3	4.8%	
	2	14	60.9%	16	25.8%	
	3	3	13.0%	6	9.7%	
LVI	4	0	0.0%	37	59.7%	0.025
	No	12	66.7%	18	36.0%	
Recurrence	yes	6	33.3%	32	64.0%	0.397
	No	11	50.0%	28	60.9%	
	yes	11	50.0%	18	39.1%	

*Chi Square Test ^Mann-Whitney U test N/A : Not Available

Table 3. Factors associated with Recurrence in luminal B breast cancer patients (n=85)

Variable		No Recur		Recurrence		p-value*
		N	(%)	N	(%)	
Age	(Median, IQR)	49.0	13.0	44.0	16.0	0.062^
Ethnicity	Malay	37	94.9%	27	93.1%	0.574
	Chinese	2	5.1%	2	6.9%	
Menopausal	pre	19	48.7%	19	65.5%	0.168
	post	20	51.3%	10	34.5%	
Parity		2.00	3.00	3.00	2.00	0.157^
HER2	pos	34	87.2%	24	82.8%	0.611
	neg	5	12.8%	5	17.2%	
	Ki67 (n=10)	0.23	0.09	0.24	0.09	0.548
Breast Affected	left	22	56.4%	12	41.4%	N/A
	right	16	41.0%	15	51.7%	
	Bilateral	1	2.6%	2	6.9%	
Tumor Size (cm)		2.70	1.40	3.00	1.30	0.437^

Variable		No Recur		Recurrence		p-value*
		N	(%)	N	(%)	
Lymph Nodes Involvement	<2	21	53.8%	15	51.7%	0.862
	2 and more	18	46.2%	14	48.3%	
Cancer Grade	1	4	10.3%	6	20.7%	0.282^
	2	18	46.2%	13	44.8%	
	3	17	43.6%	10	34.5%	
Nodular Metastasis	No	34	87.2%	19	65.5%	0.033
	Yes	5	12.8%	10	34.5%	
Lung Metastasis	No	27	69.2%	19	65.5%	0.746
	Yes	12	30.8%	10	34.5%	
Bone Metastasis	No	28	71.8%	21	72.4%	0.955
	Yes	11	28.2%	8	27.6%	
Liver Metastasis	No	29	74.4%	26	89.7%	0.113
	Yes	10	25.6%	3	10.3%	
Other Metastasis	No	32	82.1%	27	93.1%	0.183'
	Yes	7	17.9%	2	6.9%	
Cancer Stage	1	6	15.4%	2	6.9%	0.483^
	2	15	38.5%	13	44.8%	
	3	5	12.8%	2	6.9%	
	4	13	33.3%	12	41.4%	
LVI	No	16	50.0%	10	37.0%	0.318
	yes	16	50.0%	17	63.0%	

*Chi Square Test 'Fisher Exact test ^Mann-Whitney U test N/A : Not Available

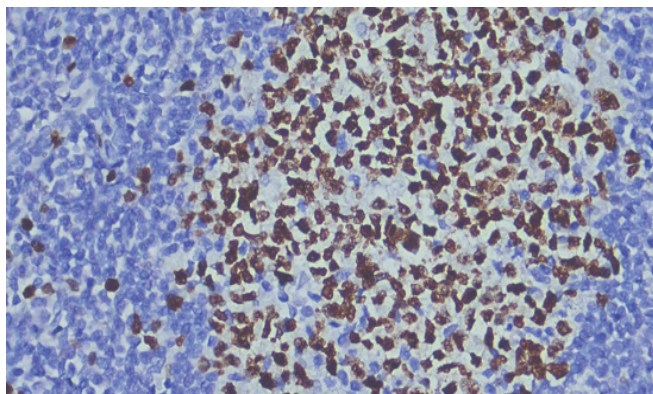


Figure 1. Ki67 positive control slide prepared from tonsil tissue at 40x magnification.

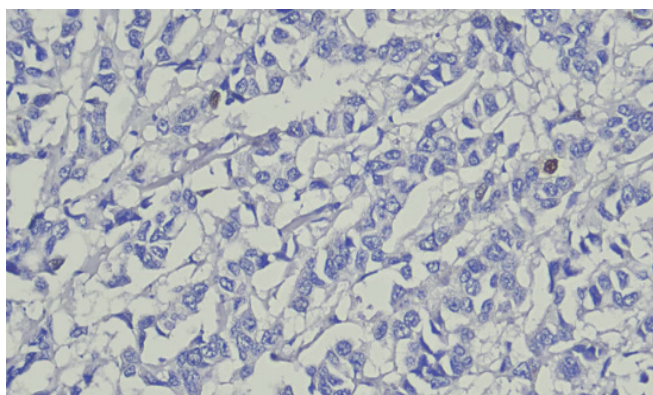


Figure 2. Ki67 stained negative slide of breast cancer tissue at 40x magnification

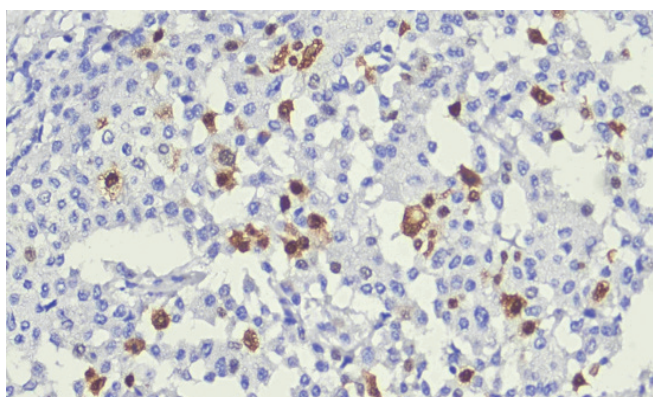


Figure 3. Ki67 stained positive slide of breast cancer tissue at 40x magnification

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