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PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

Carina Batista Paiva

ASSOCIAÇÃO DA EXPRESSÃO DO Ki67 e LINFONODOS AXILARES METASTÁTICOS COM A SOBREVIDA LIVRE DE DOENÇA NO CÂNCER DE MAMA INVASIVO

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Dissertação apresentada ao Programa de Pós-graduação em Patologia do Centro de Ciências da Saúde para obtenção do Grau de Mestre em Patologia, com orientação da professora Dra Paloma Lys de Medeiros e co-orientação do professor Dr. Paulo Roberto Cavalcanti Carvalho da Universidade Federal de Pernambuco

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“... Quem é incapaz de construir hipóteses, jamais será cientista.”

Antonio Gramsci

RESUMO

Marcadores biológicos são requeridos para estimar o risco de recorrência da doença em mulheres com câncer de mama. O Ki67 é um marcador de proliferação celular e seu valor preditivo e prognóstico tem sido estudado como um importante biomarcador de rotina na prática clínica. Nosso estudo teve como objetivo avaliar a relação entre a intensidade da expressão do Ki-67 e o envolvimento de linfonodos axilares com metástases com a sobrevida livre de doença em mulheres com câncer de mama. Esse estudo foi retrospectivo e 134 pacientes foram elegíveis para o estudo. A associação entre os escores do Ki67 e outros fatores prognósticos, tais como tamanho do tumor, grau, status dos linfonodos axilares, invasão perineural e linfovascular, biomarcadores RE, RPg e HER-2 foram avaliados, além da análise de sobrevivência. Dos 134 pacientes, 53 tiveram recorrência da doença. O valor da mediana dos escores do Ki67 foi de 30% de todos os pacientes. Um total de 78 (58,25) dos pacientes tiverm escores alto do Ki67. A associação estatisticamente significante foi entre os escores altos do Ki67 com o grau histológico ($p<0.001$), subtipos moleculares ($p<0.001$), receptores de estrógeno ($p=0.023$) e receptores de progesterona ($p=0.001$). A sobrevida livre de doença em indivíduos com Ki67 alto e axila clinicamente negativa apresentou um menor tempo em meses em comparação com os indivíduos com escores do Ki67 baixo ($p=0.049$).

Palavras-chaves: Câncer de Mama. Antígeno Ki-6. Intervalo Livre de Doença. Linfonodos.

ABSTRACT

Biological markers are required to estimate the risk of disease recurrence in women with breast cancer. The Ki 67 is a cell proliferation marker and its predictive and prognostic value has been studied as an important marker in routine clinical practice. Our study aimed to evaluate the relationship between the intensity of Ki67 expression and involvement of axillary lymph nodes with metastases to the disease-free survival in women with breast cancer. This was a retrospective study and 134 patients were eligible for the study. The association between the scores of Ki67 and other prognostic factors such as tumor size, grade, status of axillary lymph nodes, perineural invasion and lymphovascular, in addition to biomarkers ER, PgR, and HER2 were evaluated, as well as survival analysis. Of the 134 patients, 53 have had recurrence of disease. The median value of Ki scores 67 was 30% of all patients. A total of 78 (58.25) of the patients had high scores Ki 67. A statistically significant association was between high scores of Ki67 with the histological grade, molecular subtypes of progesterone receptors estrogen receptors. A statistically significant association was between high scores of Ki67 with the histological grade ($p < 0.001$), molecular subtypes ($p < 0.001$), estrogen receptor ($p = 0.023$) and progesterone receptors ($p = 0.001$). The disease-free survival in patients with high Ki67 and clinically negative axilla had a shorter time in months compared to individuals with low Ki67 scores ($p = 0.049$).

Keywords: Breast Neoplasms. Antigen Ki67. Disease-Free Survival. Lymph Nodes.

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LISTA DE ABREVIATURAS E SIGLAS

ACS	American Cancer Society
AJCC	American Joint Committee on Cancer
ASCO	American Society of Clinical Oncology
CEP	Comitê de Ética e Pesquisa
CI	Intervalo de Confiança
CNS	Conselho Nacional de Saúde
DFS	Sobrevida Livre de Doença
ESMO	European Society for Medical Oncology
ER	Receptor de Estrógeno
FISH	Hibridização in situ por fluorencência
HCP-PE	Hospital de Câncer de Pernambuco
HER2	Receptor do Fator de Crescimento Epidermal Humano
INCA	Instituto Nacional de Câncer
IHQ	Imunohistoquímica
LVI	Invasão linfovascular
OMS	Organização Mundial de Saúde
PNI	Invasão Perineural
PR	Receptor de Progesterona
SAME	Serviço de Atendimento Médico
SCN	Sistema de Classificação de Nottingham
TCLE	Termo de Consentimento Livre e Esclarecido
TNM	Tumor- Linfonodos- Metástases

SUMÁRIO

1. INTRODUÇÃO

O câncer de mama é a neoplasia de maior ocorrência entre as mulheres sendo a causa mais prevalente de morte por esta doença em todo o mundo (ACS, 2016; INCA, 2016; DIELI-CONWRIGHT et al., 2014; FEITOSA et al., 2012). Tem seu quadro agravado pelo fato do diagnóstico ser estabelecido, na maioria das vezes, numa fase tardia da doença, que diminui a possibilidade de cura do paciente. Este atraso no diagnóstico pode ser reflexo da inexistência de uma política pública consistente de controle da doença. Dessa forma, quando a neoplasia é diagnosticada nos estágios iniciais apresenta um bom prognóstico. Por isso, o diagnóstico precoce seguido de um tratamento efetivo são medidas de fundamental importância para a redução da mortalidade desta doença (INCA, 2016; MARMOT et al., 2013; KULIE et al., 2011; KRAG et al., 2010; REDIG et al., 2010).

As estimativas da Sociedade Americana de Câncer (ACS) para 2016 foram 249.260 novos casos de câncer de mama em mulheres, com 40.450 casos de morte para este ano (ACS, 2016). Para o ano de 2016 o Instituto Nacional de Câncer (INCA) estimou 596.000 novos casos de câncer no Brasil. Para o câncer de mama em mulheres foram estimados 57.960 novos casos (28,1%), com risco de 56,20 casos por 100 mil mulheres. Na região Nordeste foi previsto 11.190 novos casos (INCA, 2016).

A história natural do câncer de mama indica que o curso clínico da doença e a sobrevida variam de paciente para paciente. Essa variação é determinada por uma série complexa de fatores, tais como a diferença na velocidade de duplicação tumoral, o potencial de metástase do tumor, além de outros que estão relacionados com a condição imunológica, hormonal e nutricional do paciente (MARMOT et al., 2013). Uma melhor compreensão de moléculas chaves que estão envolvidas na patogênese do câncer de mama é essencial para um tratamento personalizado. Por isso, um número de fatores prognósticos e preditivos incluindo linfonodos axilares positivos, tamanho do tumor, grau histológico, negatividade dos receptores hormonais, positividade do fator de crescimento epidérmico humano tipo-2 (HER-2) têm sido descritos em pacientes com câncer de mama (KONTZOGLOU et al., 2013). Dessa forma, fatores prognósticos são parâmetros possíveis de serem mensurados no momento do diagnóstico e que servem como preditor de sobrevida.

O *status* do linfonodo axilar é um importante fator que determina o estágio de progressão da doença e recidiva, sendo um fator de prognóstico para doença loco regional,

preditor de sobrevida do paciente e indicação de terapia adjuvante como quimioterapia e radioterapia (INIC et al., 2014). Assim, metástases em linfonodos regionais representam a primeira etapa de disseminação do tumor em pacientes com câncer de mama, podendo as células se propagar para outros órgãos, desenvolvendo um tumor secundário. Os tecidos mais prevalentes para ocorrência de metástases são o ósseo, fígado e pulmão. (COSO et al., 2012; SINICROPI et al., 2012; WILKERSON et al.,2010; KARPANEN e ALITALO, 2008).

Marcadores tumorais, tais como o Ki67 tem sido investigado para ser avaliado como fator preditivo de metástases à distância no câncer de mama. O antígeno nuclear Ki67 tem sido usado como biomarcador de proliferação celular. O índice de proliferação celular alto– Ki67 tem sido correlacionado com a idade jovem, tumores com tamanhos maiores, linfonodos axilares positivos, receptores hormonais negativos, HER-2 positivo e pior prognóstico da neoplasia mamária. Com isso, a atividade proliferativa indicada por este biomarcador pode refletir o comportamento agressivo do carcinoma mamário e predizer o tempo de recorrência da doença identificando pacientes de alto risco de recidiva e a terapia apropriada para o tratamento (SANCHEZ-ROVIRA et al., 2012; NISHIMURA et al, 2010 e GOLDHIRSCH et al., 2009).

Diante do exposto, a identificação de marcadores tumorais específicos que possam predizer o risco de metástases para órgãos e dessa forma identificar o nível de agressividade do tumor, tem sido motivo de pesquisas. Assim, consegue-se identificar e estratificar pacientes com alto ou baixo risco para desenvolvimento de metástases e consequentemente avaliar o seu prognóstico quanto à sobrevida livre de doença. Além disso, a identificação destes biomarcadores pode ser alvo de estudo para o desenvolvimento de terapias alvos e consequentemente aumentar a sobrevida livre de doença.

2. REVISÃO DE LITERATURA

2.1 Câncer de mama

As neoplasias de mama são classificadas histologicamente de acordo com a localização de origem do tumor. O seu desenvolvimento envolve a progressão através de uma série de processos intermediários, iniciando com uma hiperproliferação ductal, seguido de uma subsequente evolução para carcinoma “in situ”, carcinoma invasivo e com o avanço da doença podendo ocorrer à metástase para outros locais do corpo. Dentre os tumores malignos, os carcinomas ductais invasivos representam o maior grupo, correspondendo a 80% dos carcinomas mamários e os lobulares estão entre 10 a 15% (HIRATA et al, 2014**)**.

Os cânceres de mama são classificados histologicamente de acordo com a localização de origem do tumor. Sendo classificados em O seu desenvolvimento envolve a progressão através de uma série de processos intermediários, iniciando com uma hiperproliferação ductal, seguido de uma subsequente evolução para carcinoma “in situ”, carcinoma invasivo e metástase. Os tumores ductais representam 80% dos casos e os lobulares estão entre 10 a 15% (HIRATA et al, 2014**)**.

A classificação molecular do carcinoma de mama segundo o perfil imunohistoquímico é baseada na avaliação dos seguintes biomarcadores: receptores de estrógeno (RE); receptores de progesterona (RPg); superexpressão do HER-2 ou amplificação do oncogene receptor do fator de crescimento humano epidérmico tipo-2 (HER-2) pela Hibridização “in situ” por fluorescência (FISH) e o antígeno nuclear Ki67 (GOLDHIRSCH et al,.2011; PICAART-GEBHART, 2011; POLYAK, 2007 e KREIKE et at., 2007).

De acordo com St. Gallen Consensus de 2011 a classificação do câncer de mama é dividida em cinco subgrupos moleculares com diferentes prognósticos e respondendo a diferentes tipos de terapias adjuvantes que são as seguintes: quimioterapia; radioterapia; hormonioterapia e terapia alvo. Sendo assim, segue a classificação em: a) Luminal A que apresenta a expressão dos seguintes marcadores, receptor de estrógeno positivo e/ou receptor de progesterona positivo; expressão do HER-2 negativo e índice de proliferação celular com baixa intensidade Ki-67≤ 14%, apresentando melhor prognóstico; b) Luminal B (HER-2 negativo) com seguinte perfil, RE positivo e/ou RP positivo, expressão do HER-2 negativo e Ki-67 > 14%; c) Luminal B (HER-2 positivo) apresentando RE positivo e/ou RP positivo e

HER2 positivo; d) HER2 positivo (não-luminal) com HER2 positivo e RE negativo e RP negativo e o e) Triplo Negativo que é definido pela falta de expressão dos seguintes marcadores (RE, RP, HER-2) com o pior prognóstico (MORRISON et al., 2012; GOLDHIRSCH et al,. 2011; PRAT et al., 2010).

O câncer de mama apresenta uma heterogeneidade tumoral, isso quer dizer que os tumores de mama com os mesmos tipos histológicos, estádios e graus de diferenciação podem apresentar desfechos distintos em relação aos fatores prognósticos, como por exemplo, sobrevida livre de doença, metástases e também diferentes respostas aos tratamentos instituídos. Uma vez que o mesmo tipo histológico, pode apresentar padrões moleculares diferentes de acordo com os biomarcadores como o receptor de estógeno, progesterona e o receptor do fator epidermal humano tipo 2 (HER-2). As neoplasias mamárias apresentam alto poder de propagação, caracterizada pela proliferação celular desordenada, sendo essas células anormais, originadas de células normais, portanto representa uma multiplicação maligna das células epiteliais que revestem os ductos ou lóbulos mamários (HIRATA et al., 2014).

2.2 Fatores de Risco para o câncer de mama

O desenvolvimento do câncer de mama envolve uma etiologia multifatorial como aspectos genéticos, ambientais, relacionados ao estilo de vida, obesidade e a hormônios específicos tais como os sexuais e insulina. Tais fatores estão associados à proliferação de células mamárias podendo evoluir para uma neoplasia maligna (MANALOPOULOS et al., 2010).

As mulheres classificadas como alto risco para o desenvolvimento do câncer de mama apresentam as seguintes características: história familiar de câncer de mama; predisposição genética, que respondem por 3% a 10% dos casos, que são as mutações nos genes BRCA1 e BRCA2 (presentes em 80% a 90% dos casos hereditários); irradiação no tórax antes do 30 anos de idade; história de carcinoma lobular “in situ” ou hiperplasia atípica (NELSON et al., 2014).

2.3 Epidemiologia

O câncer de mama é o segundo câncer mais comum em todo o mundo, sendo o mais frequente entre as mulheres com 1.67 milhões de novos casos diagnosticados em 2012, correspondendo a 25% de todos os casos de câncer. Sua distribuição em regiões pouco desenvolvidas correspondeu a 883.000 casos e em regiões desenvolvidas 794.000 casos,

sendo a quinta causa de morte por câncer com 522.000 casos em 2012 (FERLAY, J., et al.2012). A neoplasia maligna é um problema de Saúde Pública mundial, onde 20 milhões de pessoas no mundo tem câncer e a incidência no mundo cresceu 20% na última década. O impacto desta doença na população corresponderá a 80% entre os países em desenvolvimento dos mais de 20 milhões de casos novos estimados para 2025 e para o ano de 2030 foram estimados 27 milhões de casos novos de câncer. Por ser um tratamento de alta complexidade reflete em um alto impacto sócio-ecomômico para o país, dessa forma o controle da doença depende de políticas públicas e o envolvimento da sociedade. Um grande percentual dos casos de câncer (60%) é diagnosticado em estado avançado. No Brasil é a segunda causa de morte correspondendo a 190 mil por ano, sendo evitável um terço dos casos de câncer. Para o ano de 2016 foi estimado 596 mil novos casos de câncer, sendo 214.350 (51%) para os homens e 205.960 (49%) para as mulheres. Para as neoplasias malignas de mama foram estimados 57.960 novos casos, correspondendo a 28,1% de todos os casos de câncer. (tabela 1) Na região nordeste foi estimado 11.190 novos casos para 2016 (tabela 2) (INCA, 2016).

Tabela 1- Estimativa de casos novos de câncer em mulheres no Brasil referente ao ano de 2016

Colocação	Localização Primária	Casos Novos	Percentual (%)
1º.	Mama Feminina	57.960	28,1%
2º.	Cólon de Reto	17.620	8,6%
3º.	Colo de Útero	16.340	7,9%
4º.	Traquéia, Brônquio e Pulmão	10.890	5,3%
5º.	Estômago	7.600	3,7%

FONTE: Adaptado INCA, 2016.

Tabela 2- Estimativa de casos novos de câncer em mulheres no Nordeste referente ao ano de 2016

Colocação	Localização Primária	Casos Novos	Percentual (%)
1º.	Mama Feminina	11.190	20,5%
2º.	Cólon de Útero	5.630	10,3%
3º.	Colon e Reto	2.530	4,6%
4º.	Traquéia, Brônquio e Pulmão	2.100	3,9%
5º.	Estômago	1.940	3,6%

FONTE: Adaptado INCA, 2016.

2.4 Tratamento Cirúrgico

O Tratamento para esta patologia compreende a cirurgia que pode ser conservadora, que consta de tumorectomia e quadrantectomia) ou radical associada ou não a linfadenectomia axilar (LIEDTKE e RODY, 2015 & THOMSON et al., 2009). Atualmente, técnicas cirúrgicas, como a biópsia do linfonodo sentinela, que é o primeiro linfonodo que recebe a drenagem linfática do tumor primário, estão sendo empregadas, predizendo em 95% dos casos, o *status* da cadeia linfonodal, reduzindo os efeitos colaterais da linfadenectomia axilar (Hy-De LEE et al., 2014; PAIVA et al., 2011; SHAW, 2007).

A cirurgia conservadora da mama pode ser executada utilizando duas técnicas clássicas. A quadrantectomia é definida como ressecção de todo o setor mamário correspondente ao tumor, incluindo a pele e a fáscia do músculo peitoral maior. A tumorectomia ou lumpectomia consiste na remoção de todo o tumor com uma margem de tecido mamário livre de neoplasia ao seu redor (NIELSEN et al., 2006).

2.5 Tratamento Clínico

O tratamento clínico ou adjuvante, que é o tratamento após a cirurgia, consta de radioterapia, quimioterapia, hormonioterapia e terapia alvo. Quando a quimioterapia ou a radioterapia for realizada antes da cirurgia, o tratamento é chamado de neoadjuvante.

A quimioterapia representa o tratamento sistêmico e é o pilar da terapia medicamentosa em pacientes que não tem indicação de realizar a terapia endócrina, ou seja, a terapia hormonal, por exemplo, o citrato de tamoxifeno. A quimioterapia pode melhorar a sobrevida em muitos pacientes com câncer de mama no estágio inicial (RASTOGI et al., 2008).

A radioterapia pode ser empregada como terapia adujante, que é realizada após o tratamento cirúrgico ou neoadjuvante que é realizado antes da cirurgia. Este método é capaz de destruir células tumorais locais através de feixes de radiações ionizantes produzidas por aparelhos ou emitidas por radioisótopos naturais. Uma dose pré-calculada de radiação é expressa em *centigray* (cGy) ou *gray* (Gy) e a aplicação da dose é realizada por um determinado tempo em um volume de tecido determinado (LEE et al., 2008). A radiação também incide nas regiões de tecido normal, causando efeitos colaterais como dor, fadiga, alterações sensitivas e cutâneas, como a radiodermite (REIDUNSDATTER et al., 2011).

O tratamento clínico com terapia alvo é feito com o trastuzumabe ou herceptin, que é o seu nome comercial. Sua indicação está relacionado com mulheres que apresentam o marcador biológico HER-2 positivo. Dessa forma, a medicação através da ligação nesse receptor que se localiza na superfície das células tumorais que apresentam esse receptor superexpresso, bloqueando dessa forma o receptor (CHANG, 2007).

2.6 Fatores Prognósticos Clássicos

2.6.1 Tamanho do Tumor

O tamanho do tumor é um dos fatores de prognóstico mais importante, tumores menores que 1,0 cm têm entre 10% a 20% de possibilidade de comprometimento linfonodal. Contudo, tumores menores que 1,0 cm com linfonodos axilares negativos têm uma sobrevida livre de doença de 10 anos em 90% dos casos.

2.6.2 Status Linfonodal

O status do linfonodos refere se a axila apresenta linfonodos com células cancerígenas indicando clinicamente que a axila é positiva e nos casos que não há células neoplásicas nos linfonodos axilares é chamado de axila negativa. Assim, o status linfonodal é um indicador importante na sobrevida livre de doença no câncer de mama (FITZGIBBONS et al., 2000).

2.6.3 Grau Histológico

A graduação histológica Scarff-Bloom-Richardson modificado por Elston-Ellis conhecida como Sistema de Classificação de Nottingham (SCN) é o sistema de graduação recomendado por vários organismos profissionais a nível internacional como a Organização Mundial de Saúde (OMS), American Joint Committee on Cancer (AJCC) e do Royal Colégio de Patologistas (RCPath UK). A graduação histológica apresenta os escores 1, 2 e 3. O grau histológico 1 tem melhor prognóstico. Pacientes que apresentam esse escore associados com tumores menores que 2,0 cm, apresentam excelente prognóstico, com 99% de sobrevida livre de doença em 5 anos, mesmo com linfonodos axilares positivos (RAKHA et al., 2008).

2.7 Biomarcadores Tumorais no Câncer de Mama

2.7.1 Receptores de Estrógeno (RE)

Tumores RE- positivos usam esteroide hormonal estradiol como seu principal estímulo para crescimento, dessa forma, pacientes com receptores de estrógeno positivo são alvos diretos das terapias endócrinas. Estudos confirmam que os pacientes com doença RE-negativo não têm nenhum benefício durante 5 anos fazendo uso de terapia anti-hormonal, como por exemplo o citrato de tamoxifeno. Porém, aqueles que apresentam RE- negativo, mas são tumores que expressam o receptor de progesterona apresentam benéficios com o tratamento. O tratamento com anti-hormônio reduz a taxa anual de morte por câncer de mama em 31% na doença RE- positivo (FERRERO-POUS, 2000).

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2.7.2 Receptores de Progesterona (RPg)

A expressão do Receptor de progesterona é fortemente dependente da presença do RE. Tumores expressando o RPg mas não o RE são incomuns e representam 1% de todos os casos de câncer de mama. Existem os casos de câncer que apresentam apenas expressão do RPg, esses casos são raros e o tratamento com tamoxifeno apresentam pouco benefícios quanto a sobrevida livre de doença (FERRERO-POUS, 2000).

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2.7.3 Receptor do Fator de Crescimento Epidérmico Humano tipo 2 (HER-2)

O receptor do fator de crescimento epidérmico tipo 2 pertence à família dos receptores de crescimento de membrana. O gene HER-2 conhecido também como C-ErbB2 ou ERBB2; é um oncogene localizado no cromossomo 17, que é amplificado em 25% a 30% dos carcinomas de mama. Codifica uma proteína de membrana das células tumorais fazendo com que estas se desenvolvam mais rápido e aumentem a sua duplicação, tornando os tumores mais agressivos. A presença do HER-2 está associado a maior risco de recidiva tumoral e à sobrevida dos pacientes (FERRERO-POUS, 2000). A amplificação do HER-2 ou superexpressão são reconhecidas como importantes marcadores de doença agressiva e são alvos moleculares para terapias específicas, como trastuzumabe e lapatinib. (FERRERO-POUS, 2000).

2.7.4 Proteína Nuclear Ki-67

O Ki67 é um antígeno nuclear, ou seja, é uma proteína nuclear que não é expressa nas células em repouso (G0), mas pode ser detectada na fase G1, S, G2 e apresenta um pico durante a fase de mitose do ciclo celular e são baixos nas fases G1 e S.

O Ki67 é uma proteína que em humanos é codificada pelo gene MKI67 (REYAL, 2012). O escore do Ki-67 é mensurado em secções histológicas pelo método Imunohistoquímico sendo definido como um percentual de coloração de células do carcinoma invasivo (REYAL, 2012). Sua utilização como um marcador de proliferação celular mostrou que o percentual de células positivas para Ki-67 pode ser usado para estratificar pacientes com bom prognóstico e pior prognóstico. O anticorpo monoclonal MIB-1 reconhece o Ki-67.

A proteína Ki-67 é universalmente expressa entre as células em proliferação e ausente em células em repouso o que levou à maior avaliação do Ki-67 como um marcador de proliferação. O Ki-67 é um marcador de proliferação celular e um biomarcador de um subtipo intrínseco especifico do carcinoma de mama, que diferencia o subtipo molecular Luminal A (Ki-67 >14% de positividade nas células tumorais) do subtipo Luminal B, o mesmo é associado com a recorrência do câncer de mama e morte (SELZ et al., 2012; GOLDHIRSCH, 2011).

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar a relação entre a intensidade da expressão do Ki67 e o envolvimento de linfonodos axilares com metástases com a sobrevida livre de doença em mulheres com câncer de mama.

3.2 Objetivos Específicos

- Associar a intensidade da expressão do Ki67 com indicadores prognósticos como o grau histológico, invasão linfovascular, infiltração perineural e tamanho do tumor;
- Correlacionar a intensidade da expressão do Ki67 com a expressão dos biomarcadores HER-2 e os receptores hormonais;

- Associar a intensidade da expressão do Ki-67 com o status dos linfonodos axilares;
- Analisar a associação do tempo da sobrevida livre de doença com a expressão do biomarcador Ki-67;
- Analisar a associação do tempo da sobrevida livre de doença com a expressão do biomarcador Ki-67 e o status dos linfonodos axilares.

4. MATERIAIS E MÉTODOS

4.1 Desenho e Tipo de Estudo

O estudo foi realizado através do método analítico, observacional, longitudinal do tipo retrospectivo.

4.2 Local de Estudo

A presente pesquisa foi realizada no Hospital de Câncer de Pernambuco (HCP). A técnica e a avaliação das amostras teciduais da neoplasia foram realizadas através da técnica de imunohistóquimica no laboratório de Patologia do HCP, localizado na Avenida Cruz Cabugá, 1597, Santo Amaro - Recife – PE, Brasil. Tel.: (81) 32178144. E-mail: ensinoepesquisa@hcp.org.br.

4.3 População do Estudo

A amostra foi composta por 1423 casos de câncer de mama invasivo. Todos os casos foram diagnosticados no período de 2011 e 2012.

4.4 Critérios de Elegibilidade

4.4.1 Critério de Inclusão

4.4.1.1 Apresentar tumor primário de mama no seu diagnóstico inicial;

4.4.1.2 Apresentar no exame imunohistoquímico o escore do marcador Ki67;

4.4.1.3 Pacientes submetidas à cirurgia conservadora ou mastectomia com linfadenectomia axilar;

4.4.1.4 Mulheres que foram submetidas à radioterapia axilar.

4.4.2 Critérios de Exclusão

4.4.2.1 Pacientes que realizaram quimioterapia neoadjuvante ou quimioterapia para outra doença;

*4.4.2.2 Pacientes com carcinoma de mama "*in situ"*;*

4.4.2.3 Pacientes que iniciarem o tratamento no setor e não deram continuidade;

4.4.2.4 Mulheres que apresentarem metástase para outros órgãos no seu diagnóstico inicial;

4.4.2.5 Homens com diagnóstico de câncer de mama;

4.4.2.6 Mulheres diagnosticadas com câncer de mama bilateral.

4.5 Método de Coleta

4.5.1 Triagem das pacientes

A etapa inicial do estudo foi feita através da análise de todos os 1423 prontuários das pacientes diagnosticadas com câncer de mama invasivo diagnosticadas nos anos de 2011 e 2012. Para isso, foi solicitado o número de registro das mesmas no Serviço de Registro de Câncer do HCP, para que o Serviço de Atendimento Médico-SAME pudesse disponibilizar os prontuários para a análise dos dados. Os casos de óbito, os prontuários ficam armazenados em outro setor da instituição, nesses casos foi solicitado ao arquivo morto da instituição citada. De acordo com os critérios de elegibilidade da presente pesquisa, dos 1423 casos, 134 foram selecionados para o estudo.

O estudo utilizou dados de prontuários, por isso, foi utilizado o termo de Dispensa do Termo de Consentimento Livre e Esclarecido (TCLE) de acordo com a resolução nº 466⁄12 do Conselho Nacional de Saúde (CNS) para pesquisas com seres humanos (APÊNDICE A). Os

pesquisadores envolvidos no estudo se comprometeram com o sigilo dos dados de todos os pacientes envolvidos no estudo através do Termo de Confidencialidade dos Pesquisadores (APÊNDICE B).

A etapa seguinte foi à transcrição dos dados obtidos dos prontuários para uma ficha de triagem (APÊNDICE C). Nos prontuários constavam dois exames: o anatomopatológico e o exame imunohistoquímico dos biomarcadores tumorais (receptores de estrógeno e de progesterona; HER-2 e Ki-67). Além de dados como a idade da paciente no dia do diagnóstico, tempo decorrente do tratamento primário (cirurgia) até a ocorrência de recidiva local, se a paciente tinha metástase em outros locais e se houve recidiva do câncer de mama do mesmo lado da cirurgia ou contralateralmente, o número do registro, o número do prontuário, se a paciente realizou terapias como quimioterapia, radioterapia, hormonioterapia e terapia alvo (Herceptin).

A sobrevida livre de doença foi definida como o intervalo de tempo entre a cirurgia de câncer de mama até a primeira evidência de recorrência (local, contralateral a mama ou metástase), que seria o evento. Se não houve recorrência, os pacientes foram censurados no último dia do seguimento, que foi 30 de dezembro de 2015 (TANG et al., 2015; KILICHAP et al., 2014 e QI-XING, 2014). Censurado significa que a paciente não sofreu o evento (recorrência local, contralateral ou metástase) durante o período do estudo, de modo que o tempo exato de sobrevida não é conhecido. Dessa forma, diz que o paciente foi censurado.

Para a análise da sobrevida livre de doença das pacientes diagnosticadas nos anos de 2011 e 2012, foi feito o acompanhamento das mesmas através de seus prontuários até 30 de dezembro de 2015. Durante esse período foi verificado se ocorreu recidiva da doença ou metástase, assim foi anotado o dia exato da ocorrência do evento. Dessa forma pode-se calcular a sobrevida livre de doença.

4.5.2 Divisão do Ki67 de acordo com seu percentual

As pacientes foram divididas em grupos de acordo com o percentual de expressão do Ki67, dessa forma de acordo com a pontuação de Ki67 foi dividido em três categorias sendo considerado Ki67 baixo quando o percentual fosse <14% de células tumorais Ki67-positivas; intermediário ≥ 14% e 30% ≤ células Ki67-positivas; e alta > 30% de células Ki67-positivas (QI-XING, 2014).

4.5.3 Análise dos marcadores biológicos pela técnica de Imunohistoquímica

Os biomarcadores utilizados de rotina foram os receptores hormonais de estrógeno e progesterona, o marcador de proliferação celular Ki-67 e o receptor do fator de crescimento epidérmico humano 2.

A recuperação antigênica foi realizada em tampão de citrato (T-fal Steam Cuisine 700 – 10mM, ph 9,0 e 90ºC). A amplificação da reação foi realizada no Dakoautostainer (Universal Staining System – Dako). O controle negativo foi obtido substituindo-se o anticorpo primário por solução salina tamponada.

4.5.4 Marcador Receptor de Estrógeno e Receptor de Progesterona

Os receptores hormonais são proteínas expressas no epitélio e no estroma da mama o qual se ligam a hormônios circulantes, mediando efeitos de proliferação celulares. O status dos receptores de estrógeno e progesterona são avaliados pela técnica de Imunohistoquímica. Os receptores hormonais são considerados positivos com uma expressão de RE ou RPg maior ou igual a 1% de coloração nuclear (o que corresponderia a um *All red*- tudo vermelho, com escore de 2 ou mais para proporção de células positivas para RH com qualquer intensidade de imunocoloração) (FITZGIBBONS et al., 2010; HAMMOND et al., 2010).

O anticorpo primário para o Receptor de estrógeno foi o clone/SP1 que é um anticorpo anti-humano monoclonal em diluição de 1:250.

O anticorpo primário para o Receptor de progesterona foi o clone PgR 636 que é um anticorpo anti-humano monoclonal de camundongo, em diluição de 1:80.

4.5.5 Receptor do Fator de Crescimento Epidermal Humano – HER-2 neuoncogene

O HER-2 é um receptor tirosina quinase transmembrana pertencente à família dos receptores do fator de crescimento epidermal humano (EGFR). O oncogene ERBB/HER2 está localizado no cromossomo 17q21. Este oncogene é amplificado em 20 a 30% dos cânceres de mama e é considerado um marcador de pior prognóstico (HIRATA, 2014).

A superexpressão do HER2 é confirmada com a coloração das membranas celulares em cor castanha. O anticorpo primário para o HER2 ou c-erB2 será clone SP3 anticorpo monoclonal em diluição de 1:400.

Os resultados foram analisados através da escala semi quantitativamente utilizando-se os seguintes parâmetros da tabela 3:

Tabela 3- Escala semi-quantitativa referente à imunorreatividade do biomarcador HER-2

ESCORE	CARACTERÍSTICAS
0	nenhuma célula positiva
1+	imunorreatividade de membrana, fraca e irregular em pelo menos 10% das células.
2+	imunorreatividade de membrana, de intensidade fraca a moderada, porém regular em mais de 20% das células.
3+	imunorreatividade de membrana, com forte intensidade, em pelo menos 30% das células.

*Foi considerado HER-2 positivo as colorações que apresentarem escore 3+ ou amplificação pelo método FISH.

4.5.6 Índice de Proliferação celular – Antígeno Ki-67

O índice de proliferação foi avaliado através do uso do anticorpo monoclonal Mib-1 (1:100 Dako). De acordo com St. Gallean Consensus de 2011, para câncer de mama o ponto de corte do índice de proliferação do Ki67 é de 14%, ou seja, foi considerado como baixo índice proliferativo quando apresentar (≤14%) e alto índice proliferativo, quando existe mais que 14% de coloração dos núcleos, neste caso indicando maior agressividade tumoral, para diferenciar os subtipos moleculares Luminal A e Luminal B (ZURRIDA et al., 2013 e GOLDHIRSCH et al., 2011). O índice de proliferação foi calculado pelo percentual de células do tumor mostrando coloração nuclear na maioria da área proliferativa. O escore do Ki67 foi quantificado pela contagem de pelo menos 1000 células do tumor com coloração nuclear em 10 X 40 campos de alta potência selecionados aletoriamente.

4.5.7 Avaliação Histopatológica

Os aspectos morfológicos analisados foram o grau histológico que foi baseado na Escala de Scarff-Bloom-Richardson modificada por Elston-Ellis conhecida como Sistema de

Classificação de Nottingham (SCN). Os tumores foram definidos como grau histológico 1, 2 e 3. Esse grau se baseia no percentual de diferenciação tubular, ou seja, formação tubular, avaliação do pleomorfismo nuclear e índice mitótico para cada aspecto analisado e os escores variam da pontuação de 1 a 3, conforme demonstrado na tabela 4, sendo o escore 1 indicativo de melhor prognóstico para os pacientes (AJCC, 2010).

Tabela 4- Componentes referente ao Sistema de Classificação de Nottingham

GRAU HISTOLÓGICO DE NOTTINGHAN		
Formação Tubular	GRAU	
	1	Mais de 75% – maioria do tumor
	2	Entre 10- 75%- grau moderado
	3	Menos de 10% - pouca ou nenhuma
Pleomorfismo Nuclear		
	1	Tamanho do núcleo equivale a 1-1,5 x o diâmetro da hemácia, cromatina difusa, nucléolo ausente
	2	Tamanho do núcleo equivale a 1,5- 2 x o diâmetro da hemácia, cromatina grosseira, poucos nucléolos
	3	Tamanho do núcleo maior que 2,0-2,5 x o diâmetro da hemácia, cromatina vesicular, um ou mais nucléos
Índice Mitótico		
	1	0 a 11
	2	12 a 22
	3	Acima de 23
Resultado (soma dos escores)	GRAU	DIFERENCIAÇÃO
3 a 5	1	Bem diferenciado
6 a 7	2	Moderadamente diferenciado
8 a 9	3	Pouco diferenciado

Além disso, foram analisados o *status* da cadeia linfonodal que compreende o número de linfonodos dissecados, número de linfonodos comprometidos, ou seja, contagem linfonodos axilares comprometidos por células do carcinoma mamário através de evidências

histológicas (FRITZ, 2000) e tamanho do maior foco metastático. Análise do processo de invasão linfovascular e invasão perineural. O tamanho do tumor foi mensurado e foi feito o estadiamento de acordo com o Sistema TNM (TNM, AJCC, 2010), em que a medida da neoplasia será dada pelo maior diâmetro do componente invasivo.

5. MÉTODO DE ANÁLISE

A análise estatística foi feita utilizando o teste Qui-quadrado e Exato de Fisher para avaliar a associação entre os escores do Ki67 com as variáveis idade, tamanho do tumor, tipo histológico, grau histológico, tipo molecular do câncer de mama, invasão linfovascular, invasão perineural e os receptores hormonais e HER2, sendo estatisticamente significante $p>0.05$.

Para análise da sobrevida livre de doença, foi utilizado o método de Kaplan-Meier e Logrank teste, que é um teste de significância para comparar grupos em análise univariada. As análises estatísticas foram realizadas utilizando o programa pacote estatístico SPSS para Windows (versão 22.0; IBM Corp. 2012, Armond, NY, EUA).

6. CONSIDERAÇÕES ÉTICAS

O presente trabalho foi realizado após a aprovação do Comitê de Ética em Pesquisa (CEP) do Hospital de Câncer de Pernambuco ao qual foi submetido para apreciação e parecer, obedecendo integralmente os princípios éticos estabelecidos na resolução 466/12 do Conselho Nacional de Saúde (CNS). As informações foram utilizadas única e exclusivamente para a execução do presente projeto e publicação científica. A identidade das participantes será mantida em sigilo absoluto. Número do CAAE: 45709315.3.0000.5205. Número do parecer: 1.097.779

7. ASSOCIATION OF EXPRESSION KI67 AND METASTATIC AXILLARY LYMPH NODES WITH THE DISEASE-FREE SURVIVAL IN BREAST CANCER INVASIVE

Introduction

Breast cancer is the most frequent neoplasia among women and comprises a major cause of death throughout the world.[1,2,3,4] However, when diagnosed in the early stages has a good prognosis. Therefore, early diagnosis followed by an effective treatment are important measures for reducing mortality from this disease.[3,5,6,7,8] Estimates from the American Cancer Society (ACS) for 2016 were about 249.260 new cases of breast cancer in women, with 40.450 deaths this year.[1] For the year 2016 the National Cancer Institute (INCA) estimated 596,000 new cases of cancer in Brazil. For breast cancer in women 57.960 new cases were estimated at risk of 56.20 cases per 100.000 women.[3]

A better understanding of key molecules that are involved in the pathogenesis of breast cancer is essential for a personalized treatment. Therefore, a number of prognostic and predictive factors including positive axillary lymph nodes, hormone receptor negativity, HER-2 positivity and tumor size has been described in patients with breast cancer.[9]

The axillary lymph node status is an important factor that determines the stage of disease progression and recurrence, as a prognostic factor for loco regional disease, patient survival predictor and adjuvant therapy indication.[10] So metastases in regional lymph nodes represent the first stage of tumor spread in patients with breast cancer, the cells can spread to other organs, developing a secondary tumor. The most common fabrics for the occurrence of metastases are bone, liver and lung.[11,12,13,14]

Thus, other markers such as Ki67 has been investigated for reviews as a predictor of distant metastasis in breast cancer. The nuclear antigen Ki67 has been used as a biomarker of cell proliferation. The high-Ki67 cell proliferation index has been correlated with young age, tumors with larger sizes, positive axillary lymph nodes, ER / PR negative, HER-2 positive and worse prognosis of breast cancer. Thus, the proliferative activity indicated by this biomarker may reflect the aggressive behavior of breast carcinoma and predict disease recurrence time identifying patients at high risk of relapse and the appropriate therapy for the treatment. [15,16,17]

Given the above, the identification of specific tumor markers that can predict the risk of metastasis to organs and thus identify the tumor aggressiveness level, has been the subject

of research. Thus, it is possible to identify and stratify patients at high or low risk of developing metastases and consequently assessing their prognosis as the disease-free survival. Furthermore, the identification of these biomarkers may be the subject of study for the development of targeted therapies and thus increase disease-free survival. This study aimed to evaluate the relationship between the intensity of Ki67 expression and involvement of axillary lymph nodes with metastases to the disease-free survival in women with breast cancer.

Patients and Methods

Features of the Patients

This retrospective study was realized at the Cancer Hospital of the Pernambuco-Brazil. In this study, data from the 1423 patients with breast cancer, for the years 2011 and 2012 were analyzed through histopathological and immunohistochemical examinations attached in their records. We used the Consent Waiver term and Informed according to Resolution No. 466/12 of the National Health Council (CNS) to human research.

After the eligibility criteria, remaining 134 cases were included in the study. Inclusion criteria were: (1) Provide primary breast tumor in its early diagnosis, (2) had complete immunohistochemistry data including estrogen receptor (ER), progesterone receptor (PgR), HER-2, Ki67, (3) Patients undergoing breast-conserving surgery or mastectomy with axillary lymphadenectomy. Exclusion criteria were: (1) Patients underwent neoadjuvant chemotherapy or chemotherapy for another disease, (2) Women with “in situ” carcinoma, (3) Patients who start treatment in the industry and did not continue, (4) Women who present metastasis to other organs in his initial diagnosis, (5) Men with breast cancer, (6) Women diagnosed with bilateral breast cancer.

All histopathological diagnostics and biomarkers by immunohistochemistry method were determined by two experienced pathologists HCP-PE Pathology Laboratory, such procedures are routinely performed in the institution. Tissue samples of breast carcinoma were included in paraffin blocks, and subjected to 2 to 3 microns cuts in a standard microtome and stained by the method of avidin-biotin-peroxidase complex mounted on silanized slides were white STARFROST end. The routine used biomarkers hormone receptors are estrogen and progesterone, Ki67 cell proliferation and the human epidermal growth factor receptor 2. Antigen retrieval was performed by incubation in moist heat in citrate buffer (T-fal Steam Cuisine 700 - 10 mM, pH 9.0 and 90ºC). The amplification reaction was performed in

Dakoautostainer (Universal Staining System - Dako). The negative control was obtained by replacing the primary antibody-buffered saline. Positive and negative controls were used for each test.

The tumor size, grade, histological type, status nodal (axillary limp nodes positive and negative), presence of lymphovascular invasion, perineural invasion, presence the distant metastasis, local recurrence or contralateral and satus of molecules (ER, PgR, HER-2 and Ki67) were acquired from the pathology data-base. The tumors were graded according to the modified Nottingham grading system (Scarff-Bloom-Richardson Scale modified by Elston-Ellis)[18] it is defined as histological grade 1, 2 and 3. Being the score 1 indicative of better prognosis for patients. This level is based on the percentage tubular differentiation, or tubular formation assessment of nuclear pleomorphism and mitotic index for each point analyzed. and scores ranging from score 1-3. The tumor size was measured according to the 2010 7th edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM)[18] for breast cancer, wherein the extent of the neoplasm will be given by the largest diameter of the invasive component. The study was approved by the Ethics Committee of Cancer Hospital Pernambuco-Brazil (Decision Number: 1.097.779).

ER, PgR, HER-2 and Ki67 detection

ER and PgR immunohistochemical staining was performed using a monoclonal anti-human ER antibody (clone, SP1) at a dilution 1:250 and a mouse monoclonal anti-human PgR antibody (clone, 636, Dako) at a 1:80 dilution. The cut-off value for a positive result was positive staining for ER and PgR in ≥ 1% of tumor cells in 10 selected tumor sub-regions.[19,20] The results were recorded as the percentage of positively-stained nuclei, and intensity was graded between 0 and 3+ as follows: a) 0 (negative results), positive staining in < 1% of the tumor cells; b) 1+, mildly distinct, positive staining in ≤ 25% of the tumor cells; c) 2+, moderately distinct, positive staining in 25%-50% of the tumor cells; and d) 3+, strong, positive staining in > 50% of the tumor cells.

The HER-2 receptor is tyrosine kinase transmembrane receptors belonging to the family of the human epidermal growth factor receptor (EGFR). The oncogene ERBB / HER2 is located on chromosome 17q21. This oncogene is amplified in 20-30% of breast cancers and is considered a marker of worse prognosis.[21] Overexpression of HER-2 is confirmed by

staining of cell membranes in brown. The primary antibody to HER-2 or c-ErbB2 monoclonal antibody is clone SP3 dilution of 1: 400. Briefly, HER-2 staining was scored as 0, 1+, 2+ or 3+; a score of 3+ was considered to be HER2 positive and 0 or 1+ HER2 negative and samples with HER-2 scores of 2+ were confirmed to be HER-2 negative or HER2 positive using fluorescence *in situ* hybridization (FISH) analysis. Scoring for HER-2 immunohistochemistry and FISH was performed according to ASCO guidelines.[22]

Ki67 immunohistochemical (IHC) staining was performed using a mouse monoclonal anti-human Ki67 antibody (clone, MIB-1; Dako) at a 1:100 dilution. Ki67 score was defined as the percentage of nuclear staining positive cells (at least 1000) among the total nunmber of malignant cells counted in 10 high-power fields. The Ki67 expression was classified into 3 categories according to the score of Ki67: low, < 14% Ki67-positive cells; intermediate, ≥ 14% and ≤ 30% Ki67-positive cells; and high, > 30% Ki67-positive cells.[23]

The cases of the breast cancer were divided into five subtypes based on the expression levels of ER, PgR and HER-2, and the Ki67 proliferation index as follows: 1) Luminal A subtype: ER and/or PgR-positive, HER-2 negative and low Ki67 proliferation index of ≤ 14%; 2) Luminal B subtype: ER e /or PgR positive, HER-2 negative and high Ki67 index (>14%); 3) Luminal B (HER-2 positive) subtype: ER and/or PgR positive, HER2 positive and any Ki67 index; 4) HER-2 positive (non-luminal) with HER-2 positive, entrogen receptor negative and progesterone negative and 5) Triple-negative subtype: ER negative, PgR negative and HER2 negative. According to St Gallen 2011 classification[24], which considers the Ki67 value to define luminal subtypes A and B.

All patients were followed until the date of relapse or metastases or when censored at the latest date (December 30th 2015). Disease-free survival (DFS) was defined as the time interval from breast cancer surgery to the first evidence of recurrence (local,contralateral breast, or distant). When the patient presented recurrence, it is said that there was the event. If there was no recurrence, patients were censored on the last follow-up.[23]

Statistical Analysis

Statistical analysis was performed using the chi-square test and Fisher's exact to assess the association between Ki67 scores with age, tumor size, histological type, histological grade, molecular type of breast cancer, lymphovascular invasion, invasion perineural and hormone and HER-2 receptors, being statistically significant p> 0:05.

For analysis of disease-free survival, we used the Kaplan-Meier and log rank test, which is a significance test to compare groups in univariate analysis. Statistical analyses were performed using SPSS statistical package program for Windows (version 22.0; IBM Corp. 2012, Armond, NY,USA).

Results

Patients Characteristics, Clinicopathological Features and Biomarkers Expression

In this study, in total the 134 cases were eligible. The median patient age was 52 years (range, 25-90 years), and 83,6% oh these patients were ≥ 40 years. The median follow-up duration was 35 months (range, 9-72 months). The most common histological type was the invasive ductal carcinoma (87,3%). The majority of the tumor size ranged between >2cm e <5cm (T2) (57.5%). All the patients underwent axillary dissection. In total de 67/134 cases were 50% limph nodes positive. Grade II tumors accounted for 56.7% (76/134) cases.

Immunohistologically, 77.6% of the patientes were definid as ER positive, 68.7% were PgR positive and 20.1% HER2 positive. The median Ki67 score was 30% (range, 5-100%). Overall, the Ki67 scores were as follows: low Ki67 (< 14%) in 25 (18.7%) cases; intermediate Ki67 (14%-30%) in 31,1 (23.1%) cases and high Ki67 (> 30%) in 78 (58.2%).

Of the 134 eligible patients, 20 (14.9%), were classified with a Luminal A subtype; Luminal B (48.5%); Luminal B with HER2 positivo (15.7%); HER2 positive (non luminal) (3.7%) e Triple Negative (17.2%). Clinical, pathological characteristics and biomarkers in this study were shown in Table 1.

Association between Ki67 scores and clinicopathological parameters

When Ki67 was categorized into low (<14%), intermediate (14%-30%) and high (>30%) level groups, the Pearson´s Chi-square and Fisher´S Exact tests were used to verify theses results, it is significant $p<0.05$. As show in table 2. Ki67-high tumors were significantly associated with histological grade ($p<0.001$), molecular subtype ($p>0.001$), estrogen receptor ($p=0.023$) and progesterone receptor ($p=0.001$). Of the 47 subjects with grade III, 38 (80.8%) patients had high Ki67. Regarding the molecular subtype those with Luminal B subtype were 40 (61.5%) cases with high Ki6, Luminal B (HER2 positive) were 16 (76.1%) cases, to negative Triple subtype were 21 (91.4%) of cases. No statistically significant associations were identified between the scores Ki67 and age (< 40 years and ≥ 40

years) (p=0.981 and p=0.454, respectively), tumor size (p=0.478), histological type (0.094), lymphovascular invasion (0.297), perineural invasion (0.906), HER-2 (p=0.608).

Table 1. Frequency of clinicopathologic characteristics and molecular markers of the study patients

CLINICAL FACTOR		n	%
Median age (P25; P75)	52(42;64)	134	100
Age<40 years		22	16.4
Age ≥ 40 years		112	83.6
Histology	Invasive Ductal	117	87.3
	Invasive Lobular	8	6.0
	Mixt	9	6.7
Histological Grade	I	10	7.5
	II	76	56.7
	III	48	35.8
Size Tumor* (cm)	T1 (<2cm)	23	17.1
	T2(>2cm e <5cm)	77	57.5
	T3(>5 cm)	34	25.4
Clinical Status Limph Nodes	Negative	67	50.0
	Positive	67	50.0
Molecular Subtype of Breast Cancer	Luminal A	20	14.9
	Luminal B	65	48.5
	Luminal B (HER2 positive)	21	15.7
	HER-2* positive (non-luminal)	5	3.7
	Triple Negative	23	17.2
LVI*	Present	99	73.9
	Absent	35	26.1
PNI*	Present	13	9.7
	Absent	121	90.3
Estrogen receptor	Positive	104	77.6
	Negative	30	22.4
Progesteron receptor	Positive	92	68.7
	Negative	42	31.3
Human epidermal growth fator receptor 2	Positive	27	20.1
	Negative	107	79.9
Disease-free survival	Yes	81	60.5
	No	53	39.5

Font: Pernambuco Cancer Hospital- Brazil. PNI: Perineural invasion, LVI: Lymphovascular invasion, (TNM)-T= Size Tumor, HER-2: Human epidermal growth fator receptor 2

Table 2. Frequency and association between the categories of Ki 67 with the clinicopathological features and molecular biomarkers of the study patients with breast cancer

CHARACTERISTICS	LOW Ki67 (25)18.7%	INTERMEDIATE Ki67 (31) 23.1%	HIGH Ki67 (78) 58.2%	p*
Age n (%) < 40 years	1 (4.5%)	6 (26%)	16 (69.5%)	**0.981**
Age n (%) ≥ 40 years	24 (21.6%)	25 (22.5%)	62 (55.9%)	**0.454**
Tumor size* n (%)				
T1 (≤2cm)	4 (17.4%)	10 (43.5%)	9 (39.1%)	**0.478**
T2(>2cm e < 5cm)	16 (20.1%)	14 (18.9%)	47 (61%)	
T3(< 5cm)	5 (14.7%)	7 (20.6%)	22 (64.7%)	
Histological type n (%)				
Ductal Invasive	20 (17,1%)	26 (22.2%)	71 (60.7%)	**0.094**
Lobular Invasive	3 (37.5%)	4 (50%)	1 (12.5%)	
Mixt*	2 (22.2%)	1 (11.2%)	6 (66.6%)	
Histological grade n (%)				
1	3 (33.3%)	6 (66.6%)	1 (0.1%)	**<0.001**
2	17 (22.1%)	21 (27.3%)	39 (50.6%)	
3	5 (10.7%)	4 (8.5%)	38 (80.8%)	
Lymphovascular invasion (%)				
Present	8 (22.4%)	5 (13.8%)	23 (63.8%)	**0.297**
Absent	17 (17.4%)	26 (26.5%)	55 (56.1%)	
Perineural invasion n (%)				
Present	3 (23.1%)	3 (23.1%)	7 (53.8%)	**0.906**
Absent	22 (18.2%)	28 (23.1%)	71 (58.7%)	
Subtype Molecular n (%)				**<0.001**
Luminal A	20 (100%)	0	0	
Luminal B	0	25 (38.5%)	40 (61.5%)	
Luminal B (HER2 positive)	2 (9.6%)	3 (14.3%)	16 (76.1%)	
HER-2* positive(non-luminal)	2 (40%)	2 (40%)	1 (20%)	
Triple negative	1 (4.3%)	1 (4.3%)	21 (91.4%)	
Estrogen receptor n (%)				
Positive	22 (21.1%)	28 (26.9%)	54 (52%)	**0.023**
Negative	3 (10%)	3 (10%)	24 (80%)	
Progesterone receptor n (%)				
Positive	21 (22.9%)	27 (29.3%)	44 (47.8%)	**0.001**
Negative	4 (4%)	4 (4%)	34 (96%)	
HER-2* n (%)				
Positive	4 (14.9%)	5 (18.5%)	18 (66.6%)	**0.608**
Negative	21 (19.7%)	26 (24.3%)	60 (56%)	

Font: Pernambuco Cancer Hospital- Brazil. Classification TNM – T(size tumor); Mixt: Invasive ductal + invasive lobular; HER-2: Receptor do fator de crescimento epidermal tipo 2, Low Ki67 (<14% positive cells); intermediate Ki67 (14%-30%), high Ki67 (>30%), $p<0.05$ indicates a significant difference.

Association between Ki67 scores and clinical status lymph nodes axilary

Table 3 shows que 67 cases had positive lymph nodes status, most of which 67.1% had Ki67-high. Of the 78 patients with Ki67-high, 45 (57.7%) cases were positve axilla. However, the chi-square test establishes that no statistically significant difference between the involved axillary lymph nodes and scores Ki67.

Table 3. Frequency and association between the categories of Ki 67 with clinical satus lymph nodes axilary

CLINICAL STATUS LYMPH NODES	**INDEX Ki67**			**p valor**
	LOW n=25	INTERMEDIATE n=31	HIGH n=78	
Positive axilla n (%)	11(16.5%)	11 (16.4%)	45 (67.1%)	0.088
Negative axilla n (%)	14(20.9%)	20 (29.8%)	33 (49.3%)	

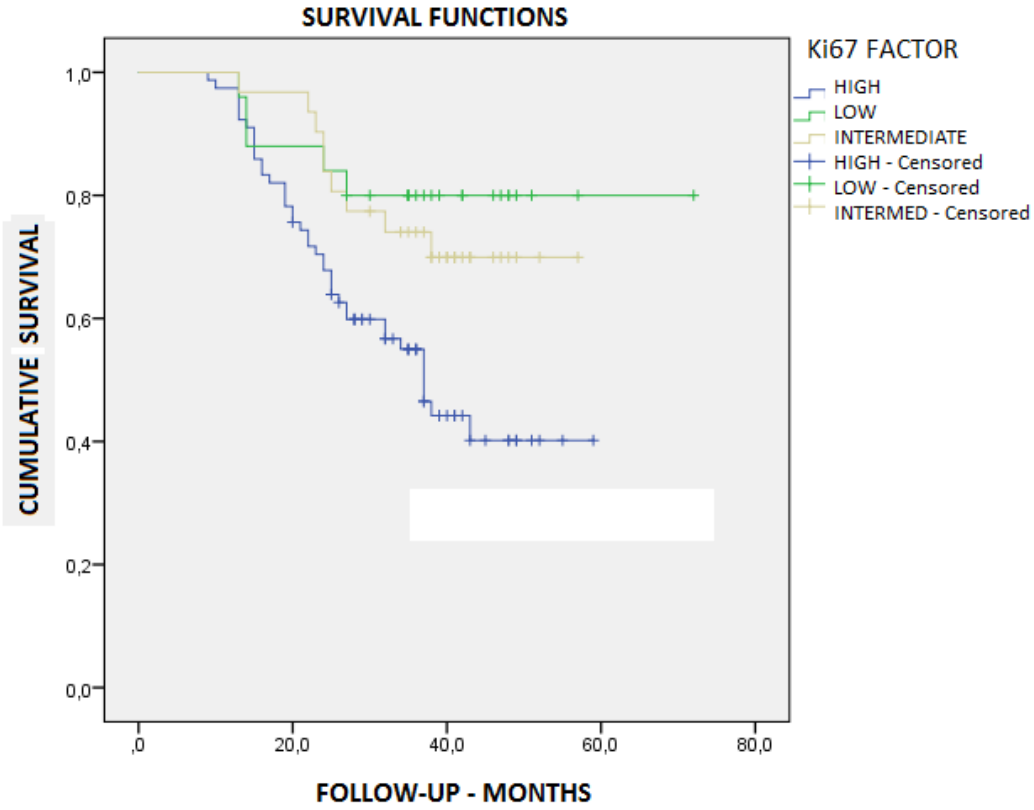
Font: Pernambuco Cancer Hospital- Brazil. Low Ki67 (<14% positive cells); intermediate Ki67 (14%-30%), high Ki67 (>30%), p<0.05 indicates a significant difference.

Survival Analysis

The median follow-up was 35 months (range, 9-72 months) among study subjects. There were 53 events related to primary cancer of the breast, these twenty-one were ipsilateral metastases breast seven contralateral breast and 27 were metastases distant, of which there were 12 cases of bone metastasis, 2 cases of brain metastases and 11 cases of metastases pulomonar.

Of the 78 patients who had the highest Ki67, 40 had a history of relapses or metastasis of the disease, accounting for 51.3% of cases. Patients with intermediate score of Ki67 (n = 31), 29% (9) of the cases presented the event and 80% (20) of the subjects who had low Ki67 were censored. Higher Ki67 levels were significantly associated with shorter disease-free survivals (DFS) compared to those os lower Ki67 levels (p=0.005) (Figure 1). In the present study subjects with high Ki67 had a DFS mean of 38.9 months (SD= 2.3) (range, 34-43 months), those with intermediate Ki67 score of 48 months (SD= 2.6) (range, 43-53 months) and low Ki67 showed an mean of 59.5 months (SD =4.5) (range, 50-68).

Figura 1. Kaplan-Meier curve disease-free survival (DFS) in each group according to Ki67 score. Log-rank test (Mantel-Cox) was significant for DFS (p=0.005).



Analysis of survival in relation to Ki 67 scores with the clinical status of the axillary lymph nodes

Lymph nodes axillary positive

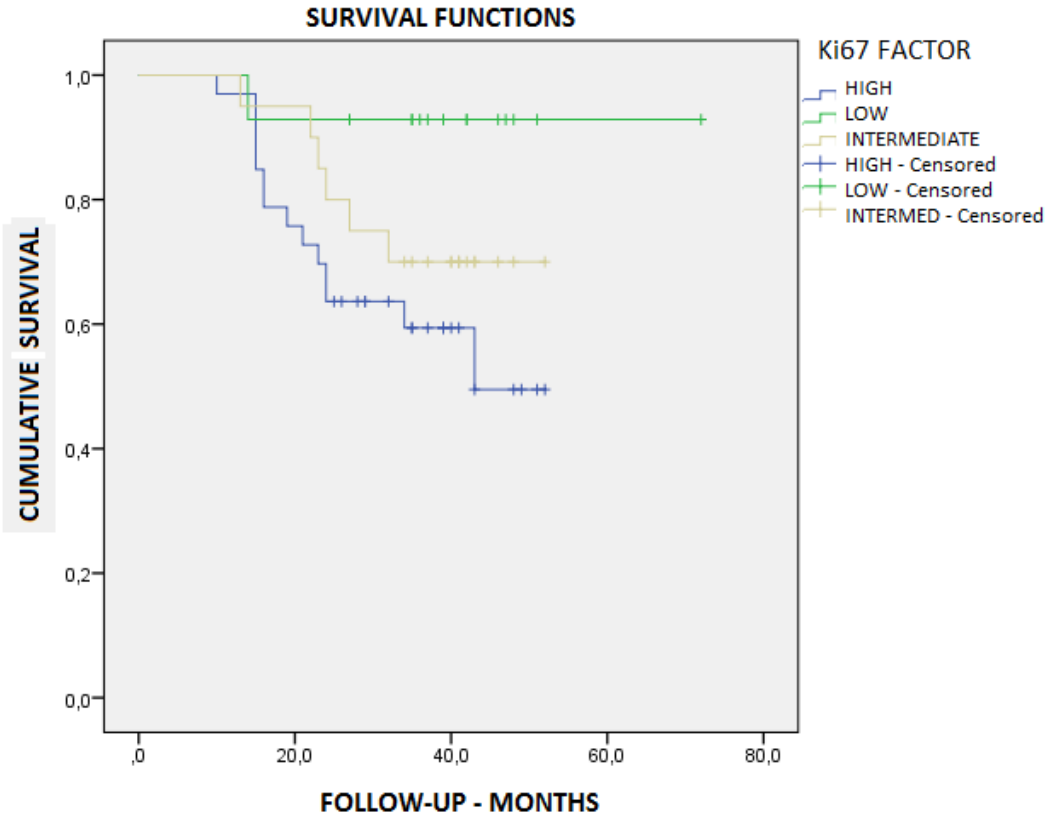
Of the 67 Patients with positive axilla, 45 cases showed a high Ki67, most of whom 25 (55.6%) cases had disease recurrence. Those with intermediate Ki67 were 11 cases, 27.3% of these had the event. Patients with low Ki67 corresponding to 11 cases only 5 had recurrence.

In the study, subjects with high Ki67 showed an average of 37 months (SD =2.8) of disease-free survival, with a 95% confidence interval (CI), being the lower limit of 31.5 months the upper limit of 42.6 months. The mean months of DFS of patients with Ki67 intermediate was 48.8 months (SD =4,0) with 40.9 months lower limit and the upper limit of

56.7 months. Those with low Ki67 had an mean of 40.9 months (SD = 5.4) of DFS with lower limit of 30.2 months and the upper limit of 51.7 months.

The Kaplan-Meier curve disease-free survival (DFS) show that subjects with Ki67-high and status limph nodes axillary positive they had a shorter time in months in disease-free survival. Nevertheless, no significant correlation was determined between the proliferative index Ki67 with satus lymph nodes axillary positive compared to disease-free survival (Figure 2).

Figura 2. Kaplan-Meier curve os disease-free survival (DFS) in subjects with positive axillary limph nodes in each group according to Ki67 score. Log-rank test (Mantel-Cox) was not significant for DFS (Chi-square test = 3.696 and p=0.158).



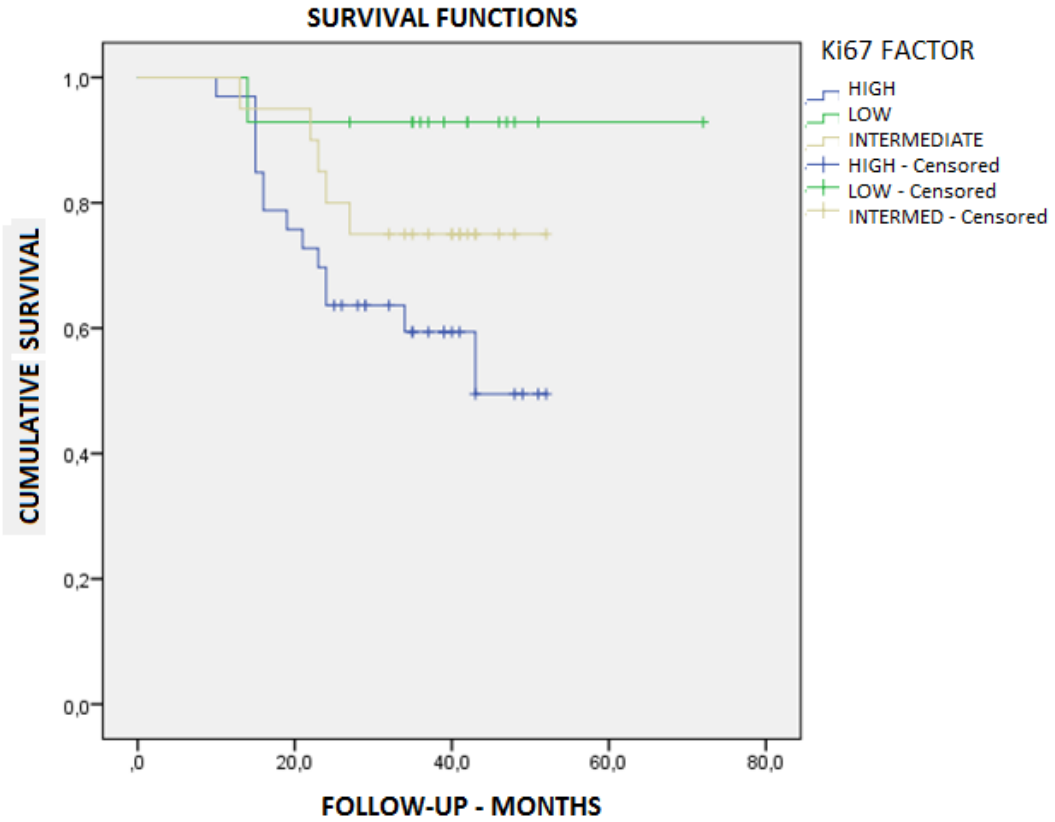
Lymph nodes axillary negative

Of the 67 Patients with axilla negative, 33 cases showed a high Ki67, of these 14 (42%) patients had recurrent disease. Those with intermediate Ki67 were 20 cases, 25% of these had the event. Patients with low Ki67 corresponding to 14 cases only 1 had recurrence.

In the study, subjects with high Ki67 showed an average of 37.9 months (SD =2.8) of disease-free survival, with a 95% confidence interval, being the lower limit of 32.2 months the upper limit of 43.5 months. The mean months of DFS of patients with Ki67 intermediate was 44.5 months (SD =2.9) with 38 months lower limit and the upper limit of 50.1 months. Those with low Ki67 had an mean of 67.9 months (SD = 3.9) of DFS with lower limit of 60 months and the upper limit of 75.7 months.

The Kaplan-Meier curve disease-free survival (DFS) show that subjects with Ki-high and status limph nodes axillary negative they had a shorter time in months in disease-free survival. Significant correlation was determined between the proliferative index Ki67 with satus lymph nodes axillary negative compared to disease-free survival (Figure 3).

Figure 3. Kaplan-Meier curve os disease-free survival (DFS) in subjects with negative axillary limph nodes in each group according to Ki67 score. Log-rank test (Mantel-Cox) was significant for DFS (p=0.049).



Discussion

Many studies have investigated the association of Ki67 expression levels and prognostic fators as histological grade, molecular subtype, estrogen receptor and progesterone receptor positive, including survival in breast cancer patients.[25,23,26,27] Kilickap et al[27] revaled that Ki67-high tumors were significantly associated with high grade (p<0.001), in this study the cutoff point for high Ki67 was> 20%. Recently, Inwald et al[28]. Demonstrated in a large cohort that Ki67 level is a importante prognostic fator for breast cancer. They reported that higher levels og Ki67 were significantly associated with lymph node positivity, higher tumor size, lymphatic invasion, ER negativity. In our study, there was a significant association between individuals histological grade 3 and high Ki67 (p <0.001). Other studies also suggest that higher Ki67 levels were positively correlated with high grade tumors.[29]

The Ki67 is highly expressed in ER negative tumor cells, its expression. Is lower in patients with ER positive tumors.[27] Also, a significant association between Ki67 expression and ER negativity was observed.[30] In another study, a significant association between higher Ki67 expression levels and ER negativity was reported.[29] The results of the present study also identified that Ki67-High was significantly associated with ER negative (p=0.023).

HER2 status is prognostic factor for breast cancer. Breast tumors which are HER2 positive tend to have higher proliferation rates. We also evaluated the relationship between Ki67 expression levels and HER2 status, and found that Ki67 expression was higher in HER2 negative patients, however these data were not statistically significant p=0.608). According to the studies of Tani et al[31]; Gunnlaugsson et al[32], there was no significant association between Ki67 expression levels and HER2 satus. On the other hand, several studies demonstrated at associatioon between higher Ki67 levels and HER2 positivity.[29,30]

As the studies of Sun et al 2015[33] results indicate an increased proliferative activity in breast cancer cells with low levels of ER and PgR, or higher Ki67 levels of HER2, and that Ki67 is na accurate biomarker that reflects tumor cellular proliferative activity.

A previous study also identified higher Ki67 expression levels in triple negative and HER-2 positive subtypes compared with luminal subtypes.[34] In accordance with our date Luminal subtypes B (HER-2 positive) and triple negative showed high expression of Ki67, being 76.1% and 91.4% respectively (p <0.001). This may be associated with subtypes of worse prognosis and more aggressive behavior as a result high proliferative activity. When comparing Ki67 levels with HER2 positive subtypes (non-luminal) and Triple negative, there

are a higher percentage of cases with high Ki67 in triple negative tumors, 20% and 91.4% respectively. This indicated the presence of stronger proliferative activity in the triple negative subtype compared with the HER2-positive (non-luminal) subtype.

The lymph node status has been intensively studied with regard to its correlatoin with the levels of Ki67. According to the studies of Azambuja 2007[35], the levels of Ki67 positivity are associated with a high risk of recurrence in both lymph node positive and negative breast cancer. In this study, 67.1% of cases with positive axillary had high Ki67, and cases with negative axilla, 49.3% had high levels of Ki67, but these data were not statistically significant ($p = 0.088$). According to the study Yin et al[36], the incidence of lymph node metastasis was higher in the high Ki67 positive group than in the low Ki67 positive group. Inic et al[37]., shows that when the Ki67 level was high, it correlated with 32 patients (94.1%) whose lymph nodes had also been involved; however when the Ki67 found to be low, it correlated with only two patients (5.9%) whose lymph nodes had also been involved (chi-square test=4.757; p=0.029).

Furthermore, disease-free survival rates were decrease in patients with higher Ki67 levels. In the study, the cutoff valeu >30% (Ki-67-high), in these cases the disease-free survival at 40 months (more than 3 years) was 45% compared with patients who had low levels of Ki67 which was almost 80% (log-rank p=0.005). However, in studies of Feng-Yan Li[38], for patients with Ki67 (> 25%) disease-free survival was 77.1% (log-rank p=0.334). In accordance with the study Qi-Xing Tan[23], a kaplan-meier analysis showed that high Ki67 labeling index strongly correlation with decreased disease-free (p=0.004), at 40 months of disease-free survival in patients with high Ki67 (> 30%) was approximately 60% compared to the subjects who had a low Ki67 (<14%) which was about 80%.

The results of this study Ki67 expression showed a positive correlation with lymph nodes negative status and disease-free survival ($p = 0.049$). For cases with negative axilla and high Ki67 in 40 months disease-free survival was about 60%. For cases with positive axillary and high Ki67 there was no statistical significance ($p = 0.158$). The studies by Paksoy et al[39], also no signicant correlation between the proliferative index Ki67 in the tumors and metastatic lymph nodes (p>0.05).

Conclusion

The present study has shown a significant association was identified between Ki67 and histological grade, molecular subtype, ER and PgR and axillary lymph node invovement. The high levels of Ki67 expression showed low disease-free survival in patients with negative

axilla. Thus, analysis of Ki67 expression may be useful in clinical pratice and may presente na otpion for the personalized treatment of breast cancer patients.

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APÊNDICES

APÊNDICE A- DISPENSA DO TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

HOSPITAL DE CÂNCER DE PERNAMBUCO

DISPENSA DO TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Venho por meio desta informar ao Comitê de Ética em Pesquisa que o projeto de pesquisa intitulado: **ASSOCIAÇÃO DA EXPRESSÃO DO ki-67 e LINFONODOS AXILARES METASTATICOS NO CANCER DE MAMA INVASIVO COM A SOBREVIDA LIVRE DE DOENÇA,** não utilizará o Termo de Consentimento Livre e Esclarecido por que a pesquisa será realizada em prontuário de pacientes. A autora da pesquisa resguarda os dados pessoais dos pacientes e informar que estes serão utilizados apenas para alcançar os objetivos do trabalho exposto acima, incluídos sua aplicação na literatura cientifica especializada e apresentação em eventos científicos.

Recife, ____ de _____ de 2015.

_____

Pesquisador Responsáve

APÊNDICE B - TERMO DE CONFIDENCIALIDADE DOS PESQUISADORES

Título do Projeto: Associação da expressão ki-67 e linfonodos axilares metastáticos com a sobrevida livre de doença no câncer de mama invasivo.

Pesquisador responsável: Carina Batista de Paiva
Instituição/Departamento: Universidade Federal de Pernambuco/ Departamento de Patologia
Telefone de Contato: (81) 8681 -6072/ (81) 9659 -2936

A pesquisadora, a orientadora e os co-orientadores do presente projeto, se comprometem a preservar a privacidade dos dados coletados e disponibilizados para a pesquisa. Os dados serão acessados exclusivamente pela equipe de pesquisadores e a informação arquivada em papel não conterá a identificação dos nomes dos sujeitos elencados. Este material será arquivado de forma a garantir acesso restrito aos pesquisadores envolvidos, e terá a guarda por cinco anos, quando será incinerado. Concordam, igualmente, que essas informações serão utilizadas únicas e exclusivamente para a execução do presente projeto. As informações somente poderão ser divulgadas de forma anônima e serão mantidas (nos computadores/arquivos das salas) dos grupos de pesquisa da (instituição envolvida) sob a responsabilidade de Carina Batista de Paiva. Este projeto está em avaliação/aprovação do Comitê de Ética e Pesquisa Envolvendo Seres Humanos, do Hospital de Câncer de Pernambuco.

Recife, _____ /_____ /_____

(Pesquisadora responsável)

(Orientadora)

(Co-orientador)

(Co-orientador)

APÊNDICE C - FICHA DE TRIAGEM PARA SELEÇÃO DOS PACIENTE

HCP
HOSPITAL
DE CÂNCER
DE PERNAMBUCO

FICHA DE TRIAGEM

1. **IDENTIFICAÇÃO**

Nº DO REGISTRO:_____Nº PRONTUÁRIO:_____

IDADE NO DIA DO DIAGNÓSTICO:_____DATA DE NASCIMENTO:___/___/___

DIAGNÓSTICO:_____

TERAPIAS ADJUVANTES:
QUIMIOTERAPIA: ( )NÃO ( ) SIM_____
RADIOTERAPIA: ( )NÃO ( ) SIM_____
TERAPIA ALVO: ( ) NÃ0 ( ) SIM_____
ANTI-HORMONAL: ( ) NÃO ( ) SIM_____

SOBREVIDA LIVRE DE DOENÇA: ( ) SIM – TEMPO:_____

( ) NÃO – TEMPO:_____ RECIDIVA LOCAL (IPSILATERAL): ( )/ METÁSTASE A DISTÂNCIA : ( )/ RECIDIVA DE CÂNCER DE MAMA CONTRA-LATERAL: ( ).

2. **ASPECTOS DO EXAME ANATOMOPATOLÓGICO - CARACTERÍSTICAS CLINCOPATOLÓGICAS.**

TIPO HISTOLÓGICO	
GRAU HISTOLÓGICO	
STAUS LINFONODAL: NÚMERO DE LINFONODOS DISSECADOS NÚMERO DE LINFONODOS COM METASTÁSE TAMANHO DO LINFONODO	
TAMANHO DO TUMOR	
INVASÃO ANGIOLINFÁTICA	**SIM ( ) NÃO ( )**
INVASÃO PERINEURAL	**SIM ( ) NÃO ( )**

OBSERVAÇÕES:

3. **RELATÓRIO TÉCNICO DE IMUNOHISTOQUÍMICO- EXPRESSÃO DOS BIOMARCADORES**

RECEPTOR DE ESTRÓGENO	**NEGATIVO ( )**	**POSITIVO ( )** **INTENSIDADE (%)** **FORTE:_____** **FRACA:_____**
RECEPTOR DE PROGESTERONA	**NEGATIVO ( )**	**POSITIVO ( )** **INTENSIDADE (%)** **FORTE:_____** **FRACA:_____**
RECEPTOR DO FATOR DE CRESCIMENTO EPIDERMAL HUMANO TIPO 2 - HER-2	**NEGATIVO ( )**	**POSITIVO ( )** **ESCORE ( )**
Índice de Proliferação Ki-67	**NEGATIVO( )**	**POSITIVO ( )** **INTENSIDADE (%)**

4. **Pesquisadora:_____.**

Recife, _____ de_____de 2015

APÊNDICE D – ARTIGO PUBLICADO

DOI: 10.1590/1809-2950/15214123032016

ORIGINAL RESEARCH

Prevalence of lymphedema after breast cancer treatment in overweight patients

Prevalência de linfedema após tratamento de câncer de mama em pacientes com sobrepeso

La prevalencia de linfedema tras tratamiento de cáncer de mama en mujeres con sobrepeso

Carina Batista de Paiva[1], Cintia Maria da Silva Dutra[1]

ABSTRACT | Breast cancer is the neoplasia with the highest incidence in the population worldwide, and lymphedema is one of the most frequent complications in the treatment. Body mass index increase is one of the risk factors for lymphedema after breast cancer treatment. The objective of this study was to verify the incidence of lymphedema in mastectomized women with overweight and obesity. The risk of lymphedema in women with overweight and obesity was four times greater (Odds Ratio, OR = 3.887). The higher the body mass index, the higher was the probability of lymphedema, with increase in the relative risk of 40% for obesity II.

Keywords | Breast Neoplasmas; Lymphedema; Obesity.

RESUMO | O câncer de mama é a neoplasia de maior ocorrência no mundo, e o linfedema é uma das complicações mais frequentes do tratamento. O aumento do índice de massa corporal é um dos fatores de risco para linfedema após o tratamento do câncer de mama. O objetivo deste estudo foi verificar a incidência de linfedema em mulheres mastectomizadas com sobrepeso e obesidade. Os resultados mostraram que o risco de linfedema em mulheres com sobrepeso e obesidade foi quatro vezes maior (Odds Ratio, OR=3,887). Quanto maior o índice de massa corporal, maior a probabilidade de linfedema, com aumento do risco relativo de 40% para obesidade II.

Descritores | Neoplasias de Mama; Linfedema; Obesidade.

RESUMEN | El cáncer de mama es la neoplasia que más ocurre en el mundo, y el linfedema es una de las complicaciones más frecuentes de su tratamiento. El aumento del índice de masa corporal es uno de los factores de riesgo para el linfedema tras el tratamiento de cáncer de mama. El propósito de este estudio fue verificar la incidencia de linfedema en mujeres sometidas a mastectomía y que están con sobrepeso y obesidad. Los resultados mostraron que el riesgo de linfedema en mujeres con sobrepeso y obesidad ha sido cuatro veces mayor (Odds ratio, OR=3,887). Cuanto mayor es el índice de masa corporal, mayor es la probabilidad de linfedema, con aumento de riesgo de 40% para obesidad II.

Palabras clave | Neoplasias de la Mama; Linfedema; Obesidad.

The research was conducted at the Cancer Hospital of Pernambuco (HCP) in the ambulatory service of breast physiotherapy – Recife (PE), Brazil.

[1]Physical therapist, HCP – Recife (PE), Brazil.

Mailing address: Carina Batista de Paiva – Rua Henrique Dias, 24, Vila Torres Galvão –CEP Paulista (PE), Brazil – CEP: 53403-485 – E-mail: carinapaiva_8@hotmail.com – Financing source: Nothing to declare – Conflict of interest: Nothing to declare – Presentation: July 2016 – Accepted for publication: Oct. 2016 – Approved by the Research Ethics Committee of the Cancer Hospital of Pernambuco: CAEE 20056113.1.0000.5205.

INTRODUCTION

Breast cancer is the neoplasia of highest incidence among women in developed or developing countries, achieving high rates of morbidity and mortality[1-4]. Estimates from the American Cancer Society (ACS) for 2016 were approximately 246,660 new cases of breast cancer in women, with 40,000 cases of death for this year[5]. According to the National Cancer Institute of Brazil (INCA), in 2016, 57,960 new cases of breast cancer were estimated in the country[2].

Among the post-operative complications of breast cancer, the most frequent is lymphedema, a chronic condition caused by accumulation of liquid rich in proteins in the interstitial space[6-10]. The lymphedema development can happen immediately after surgery in rare cases, or years after the treatment[11-14]. The lymphedema incidence varies in different studies, being observed in approximately 20% of cases. The rates may vary from 6 to 65%[15-18].

The emergence of lymphedema is multifactorial and is associated with the dissection of the axillary lymph nodes, caused by radiotherapy, obesity, surgery, recurrence of cancer in the axillary lymph nodes, infection and lymphangitis[13,19-21]. The lymphedema degree is associated with the degree of obesity, because the additional deposition of subcutaneous fat contributes to increase the arm volume and the separation of the deep lymphatic channels[22]. Weight gain after diagnosis of breast cancer is related to radiotherapy, chemotherapy and endocrine therapy[23]. Obesity is a risk factor of infection and delay of the cicatrization process, tumorous reincidence and comorbidities, among other post-operative complications such as seroma, hematoma and axillary web syndrome[24]. In addition, body mass index (BMI) increase, mainly in severe obese (BMI≥40 Kg/m^2), causes serious health problems, such as increase in the risk factor for cardiovascular, metabolic, neoplastic and orthopedic diseases[25-27].

Therefore, lymphedema is one of the main complications from the breast cancer treatment, and is associated with adverse psychosocial and physical consequences, interfering on the patient's quality of life[19]. The objective of this study was to verify the lymphedema incidence in mastectomized women with overweight and obesity.

METHODOLOGY

A descriptive, observational, transversal study, in which In total, participated 100 women who had undergone mastectomy and were under physical therapy treatment. The study was conducted from July 2013 to August 2014is at the physical therapy ambulatory of the Cancer Hospital of Pernambuco.

Data collection

The data were collected at the physical therapy ambulatory of the Cancer Hospital of Pernambuco. On their appointment days, the volunteers were invited to participate in the research. The objectives and benefits of our study were made explicit. In case of agreement, they were asked to sign the informed consent form, in accordance with resolution 466/12 from the National Health Council. After the explanation of the procedure to be performed and the signed form, the volunteers were submitted to physiotherapeutic evaluation of the upper limb perimetry.

The anthropometric assessment consisted of calculating the body mass index (BMI). BMI values between 25 and 30 were considered overweight, and BMI>30 was considered obesity[27]. An evaluation form was developed for the selection of patients. All participants had BMI classified as overweight or obesity. Among them, 53% were married, 30% single, 9% divorced and 8% widow. The average age was 52.5 years (SD=7.9).

The lymphedema assessment was performed by perimetry of morbidity rates of the upper limbs. The measurements of the circumference (in centimeters) were taken in eight points. The point of reference was the crook of the arm, to mark the measurements. The latter were taken every 7 cm, in three points below the crook of the arm, with the limb supported, relaxed and in the position of supination and every 7 cm, in three points above the crook of the arm, in addition to the circumference of the wrist and the hand. The measuring tape was placed over the marks mentioned[28]. Lymphedema was diagnosed when the circumference of one or more measurements in the affected side was 2.0 cm bigger than the circumference of the same point in the contralateral limb, according to the protocol established by the HCP of Pernambuco.

Instrumentation

Venosan brand tape was used for the perimetry evaluation. To measure body mass and height, a digital scale (Welmy) was used, reference W2005 (capacity of 200 kg) and Welmy stadiometer (200cm) (maximum height of 200cm), placed on a flat surface with good lighting. On the scale, the women were positioned erect and with heels together for the calibration of height and weight. Body mass index (BMI) was calculated by dividing the subject's mass by the square of their height. The mass was recorded in kilograms and the height in meters.

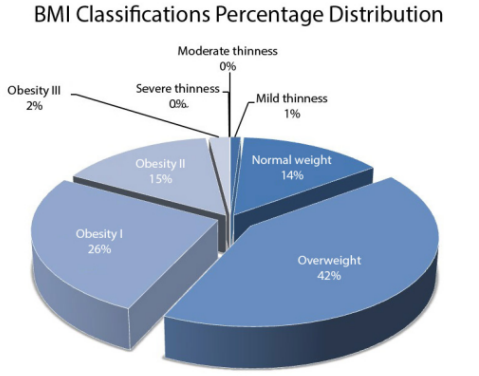
Statistical Analysis

For the statistical analysis, we carried out a simple logistic regression, with the purpose of evaluating the prevalence of lymphedema as a dependent variable, in relation to the predictive factors "overweight" and "obesity" as independent variables. Separately, the predictive factors "overweight" and "obesity level I, II and III" were tested, with the aim to know the individual influence of each factor regarding lymphedema, through the Chi-square test.

To indicate the chance of a person – who is overweight or obese – having lymphedema, the prevalence ratio, the Odds Ratio (OR), the relative risk increase (RRI) and the number needed to cause the condition (NNC) were calculated. We used Microsoft Excel, SPSS18 and EpiInfo.

RESULTS

The population under study was classified according to the parameters stipulated in the body mass index (BMI) indicated by the World Health Organization. Severe thinness (BMI<16 kg/m²): no elements present in the sample; moderate thinness (BMI between 16 and 17 Kg/m²: no elements in the sample; light thinness (BMI between 17 e 18.5 Kg/m²): one participant (1%); normal weight (BMI between 18.5 e 25 kg/m²): 14 participants (14%), overweight (BMI between 25 and 30 Kg/m²): 42 participants (42%); obesity I (BMI between 30 and 35 Kg/m²): 26 participants (26%); obesity II (BMI between 35 and 40 Kg/m²): 15 participants (15%), and obesity III (BMI≥40 Kg/m²): 2 participants (2%), as shown in Chart 1.



Graph 1. Distribution of patients according to the classification of body mass index (BMI)

According to the logistic regression analysis, the chance of lymphedema emergence in women with predictive factors (overweight and obesity) was approximately 4 times (OR=3.887; p<0.05), considering women that were submitted to the same surgical treatment, but did not present overweight or obesity. The probability for the development of lymphedema was 37.4% for women with a history of overweight and obesity, and 13.3% for those who did not have these risk factors.

The Chi-square test found no significant difference (p=0.308) in the prevalence of lymphedema in overweight individuals, and 11 of them presented lymphedema (11/42). However, the overweight patient is twice more likely to develop lymphedema (OR=2.31), with 12.9% of relative risk increase.

Among the 26 cases of obesity degree I, 12 showed lymphedema (12/26) with a probability six times higher to develop the condition, compared with individuals without the predictive factor (OR=5.57), as shown in Table 1. For every four obese patients, one individual will present lymphedema (NNC=4), with RRI of 32.8% (Table 1).

Table 1. Comparison of lymphedema occurrences among predictive factors – Chi-square tests; relative risk increase (RRI) and the number needed to cause the condition (lymphedema) (NNH)

Study	Chi-square p-Value	Odds Ratio	RI	NNC
With overweight Without overweight	0.308	2.310	12.86%	8
With obesity I Without obesity I	0.033	5.570	32.82%	4
With obesity II Without obesity II	0.020	7.420	40.00%	3
With obesity III Without obesity III	0.201	6.500	36.67%	3

Concerning women that presented obesity II (15), this variable had the highest prevalence ratio (PR=4), as shown in Table 2. There was statistical significance (p=0.020) and OR=7.42, that is, a chance seven times higher for the emergence of lymphedema compared with a patient who is not obese (Table 2).

Table 2. Values for the occurrence of lymphedema in relation to predictive factors and the prevalence ratio

Classification/ Predictive factors	n	Presence of Lymphedema		Prevalence Ratio
		Yes (positive cases)	No (negative cases)	
Severe thinness	0	0	0	0
Moderate thinness	0	0	0	0
Light thinness	1	0	1	0
Normal weight	14	2	12	0
Overweight	42	11	31	1.964
Obesity I	26	12	14	3.462
Obesity II	15	8	7	4.000
Obesity III	2	1	1	3.750
TOTAL	100	34	66	

Two cases were observed for obesity III, of which one presented lymphedema, with prevalence ratio of 3.75. The Chi-square did not reach statistical significance. The OR indicated six times more chances of developing lymphedema.

DISCUSSION

A previous study[19] reported that out of the 455 women evaluated, 124 presented lymphedema. Among these, 114 had BMI>25 Kg/m^2, that is, 91.9% of the overweight or obese women presented lymphedema[19]. People who have a higher BMI need more blood in circulation and higher efficiency of the lymphatic system to keep its flux. Possibly there is an imbalance of the transportation and absorption capacity of the lymph, which increases the risk of lymphedema[29,30].

The overweight patient is twice more likely to develop lymphedema (OR=2.31). Another study also found that the increase in the arm volume was related to BMI. It was observed that women with higher BMI tended to have a change in the upper limbs volume[13]. However, there was no stratification of overweight and obesity cases. Although the relative risk increase (RRI) has been low in this study, there is a risk to develop the condition (lymphedema): for eight patients with overweight/obesity, one will present lymphedema (NNC=8).

For the cases that presented obesity level I, the risk of developing lymphedema was about six times higher. In other studies, 79% of patients (359 women) presented BMI≥25 and 32% (145 women) had lymphedema, with OR=3.94[19]. Another study reported that 92% of the patients with lymphedema had obesity, however the authors did not specify the obesity degree [31]. Women with BMI≥30 were 3.6 times more likely to develop lymphedema in six months[32].

The reduction of body weight interferes on the arm volume reduction. The study of Shaw et al. [27] showed a reduction from 24%±12% to 15%±10%. According to Kwan et al.[16], obese women present OR= 2.34; however, in their study the relationship with the obesity degree was not investigated. Proportionally, in our study, the increased relative risk was 40%, the NNC was only 3 individuals with overweight/obesity for the emergence of a new case of lymphedema.

For the cases of obesity level III, the reduced size of the sample impaired the estimation. Ahmed et al.[6] observed that most women evaluated had BMI>30 Kg/m^2 and lymphedema. They associated this fact not only to obesity, but found that, at the time of the diagnosis, these women had larger tumors and dissection need of a bigger amount of lymph nodes. However, Demark-Wahnefried et al.[10] found that BMI≥30 was not associated with increased risk of lymphedema in the assessment 30 months after surgery.

CONCLUSION

The analysis of significance indexes, probability and epidemiological indexes of the sample and the predictive factors (overweight and obesity) showed strong interaction between overweight and obesity and the presence of lymphedema.

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ANEXOS

ANEXO A- NORMA DA REVISTA THE BREAST

THE BREAST

An Associate Journal of the Australasian Society for Breast Disease

Affiliated with the European Society of Breast Cancer Specialists

Official Journal of Breast Centres Network

AUTHOR INFORMATION PACK

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DESCRIPTION

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