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**Identificação da Expressão dos
Marcadores de Prognóstico *UGT8* e
GATA3 no Câncer de Mama**

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Dissertação submetida ao Programa de Pós-Graduação em Patologia da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre

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“O cientista não é o homem que fornece as
verdadeiras respostas; é quem faz as
verdadeiras perguntas”

(Claude Lévi-Strauss)

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LISTA DE ABREVIATURAS

IARC –	<i>International Agency for Research on Cancer</i>
INCA –	Instituto Nacional do Câncer
TNM –	Tumor primário, estado nodal e metástases à distância
G –	Grau tumoral
OMS –	Organização Mundial da Saúde
NGS –	Classificação de Nottingham
RE –	Receptor de estrógeno
RP –	Receptor de progesterona
HER-2 –	Receptor 2 do fator de crescimento epidérmico humano
Ck 5/6 –	Citoqueratina 5/6
Ki-67 –	Proteína Ki-67
<i>p53</i> –	Gene <i>p53</i>
DNA –	Ácido desoxirribonucleico
EGFR –	<i>Epidermal growth factor receptor</i> (receptor do fator de crescimento epidérmico)
<i>UGT8</i> –	Ceramida galactosiltransferase
RNA _m –	Ácido ribonucleico mensageiro
GalCer –	Galactosilceramida
GATA3 –	Gene codificador da proteína GATA-3
GATA-3 –	GATA-proteína de ligação 3
HE –	Hematoxilina e Eosina

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RESUMO

INTRUDUÇÃO: O câncer de mama é a neoplasia mais comum entre as mulheres, possui diferentes fenótipos moleculares relacionados à expressão dos marcadores tumorais. Além dos marcadores já estabelecidos pela literatura, podem-se citar outros marcadores importantes relacionados ao câncer de mama, como a *ceramida galactosiltransferase (UGT8)*; e, o *GATA3*, cujo estudo tem por objetivo propiciar um melhor entendimento sobre a doença.

OBJETIVO: Associar a expressão de *UGT8* e *GATA3* com o tamanho e grau tumoral TMN e os fenótipos moleculares em amostras de pacientes com tumor primário de mama.

MATERIAIS E MÉTODOS: Para a pesquisa, foram selecionadas pacientes com câncer de mama no ambulatório de mastologia do hospital de referência. Elas responderam a um questionário e consentiram, para pesquisa, na utilização dos blocos de anatomopatológico. A partir disto, foi realizada a imunohistoquímica dos marcadores *UGT8* e *GATA3* nas amostras e, o resultado, foi associado com TMN, estadiamento, grau e os subtipos moleculares.

RESULTADOS: Foram incluídos 174 questionários de pacientes com questões referentes a dados demográficos, vida reprodutiva e fatores de risco. Para o estudo dos marcadores, foram selecionados 121 blocos de anatomopatológicos. Em relação aos marcadores estudados, verificou-se uma associação significativa entre a expressão elevada de *UGT8* com tamanho tumoral $\leq 2\text{cm}$ ($P=0,043$) e expressão negativa com RP ($P=0,029$). A alta expressão de *GATA3* associou-se ao estadiamento I ($P=0,05$) e, sua falta de expressão foi verificada de forma significativa em tumores com estadiamento III ($P=0,05$) e tamanhos de 2,1a 5cm ($P=0,019$).

CONCLUSÃO: Com base nos resultados encontrados, pode-se concluir que houve uma associação significativa entre a expressão dos potenciais marcadores prognósticos, *UGT8* e *GATA3*, ao tamanho tumoral, expressão de RP e estadiamento das amostras estudadas.

Palavras-chave: TMN. *UGT8*. *GATA3*. Câncer de mama.

1. INTRODUÇÃO

1.1. Patogenia do câncer de mama

O câncer de mama é uma doença de característica heterogênea que se reflete na sua classificação molecular, na morfologia, no curso clínico e na sua resposta ao tratamento (Seong e cols. 2015; Van Schooneveld e cols. 2015). Essa neoplasia é uma das mais comuns entre as mulheres e corresponde à segunda causa de morte no mundo (IARC 2015; Assi e cols. 2013). Quando diagnosticado em estágios iniciais, as pacientes possuem uma sobrevida de 83% a 92%, relativa aos 5 primeiros anos após o seu diagnóstico (Brewster e cols. 2007; Tao e cols. 2015; IARC 2015; Assi e cols. 2013).

O câncer de mama tem sido considerado um problema de saúde pública, inclusive, em países desenvolvidos onde houve um aumento no número de casos da doença acompanhado de uma redução da mortalidade por essa neoplasia (Gebrim 2012; Hana Azmi 2013). Essa doença também causa preocupação em países em desenvolvimento, como o Brasil, onde a dificuldade relacionada à prevenção de maneira eficaz tem se tornado determinante para o aumento no número de mulheres acometidas pelo câncer (Gebrim 2012; Hana Azmi 2013).

Entretanto, o relevante é que nas últimas décadas a mortalidade ocasionada pelo câncer de mama vem decrescendo e esse declínio tem sido atribuído, principalmente, à abordagem multidisciplinar referente ao tratamento e à compreensão mais profunda de sua biologia (Senkus 2013; Options 2010). A sua ampla variedade faz com que cada um dos tipos de câncer de mama existente possua um comportamento biológico especial, com conduta clínica e tratamento específico,

além de diferentes prognósticos (Senkus 2013; Harbeck e Würlstein 2013; Hana Azmi 2013).

1.2. Epidemiologia

O câncer de mama é o tipo mais frequente que acomete as mulheres, no Mundo e no Brasil, porém as taxas de incidência modificam-se ao longo do tempo e da sua geografia (IARC 2012; INCA 2015; Rudel e cols. 2014). Segundo dados da *International Agency for Research on Cancer* (IARC), as incidências variam de 19,3 a cada 100.000 mulheres na África Oriental e 89,7 por 100.000 mulheres na Europa Ocidental e tem se tornado cada vez mais frequente nos países em desenvolvimento (IARC 2015). Ainda, segundo o IARC, a incidência da neoplasia em 2012 foi de 1,6 milhões de novos casos no mundo, com taxa de mortalidade de aproximadamente 13,2 a cada 100.000 pacientes, sendo sua maioria localizada na América do Norte e na Ásia (IARC 2012).

Já no Brasil, conforme informações fornecidas pelo Instituto Nacional do Câncer (INCA) o número estimado de novos casos da doença para 2015 foi de 57.120, com taxa de mortalidade de 14.207 mulheres em 2013 (INCA 2015). Neste contexto com exceção dos tumores de pele não melanoma, o câncer de mama apresenta maior prevalência, respectivamente, nas regiões Sudeste (30.740), Nordeste (10.490), Sul (10.370) e Centro-Oeste (3.800) (INCA 2015). Atualmente, o Rio Grande do Sul é o estado da região sul com maior incidência de neoplasia, correspondendo a 87,72 novos casos a cada 100.000 mulheres, inclusive superando a incidência dos tumores de pele não melanoma (INCA 2015).

A epidemiologia do câncer de mama tem sido intensamente estudada ao longo do tempo, pois sua incidência tem aumentado a cada ano dentro da população feminina (Trichopoulos et al. 2008; Stuckey 2011).

1.3. Fatores de Risco

As alterações no estilo de vida, bem como a adoção de hábitos não saudáveis da população podem contribuir para o aumento de casos de câncer na população mundial (Mohite e cols. 2015). Segundo alguns estudos, a mudança relacionada ao estilo de vida das pacientes contribui de forma significativa para o desenvolvimento da neoplasia mamária (Mohite e cols. 2015; Ekelund e cols. 2015; Di Cello e cols. 2013; Peeters e cols. 2014).

Atualmente existem alguns fatores de risco que são importantes no processo de desenvolvimento da neoplasia, dentre eles, Mohite e cols (2015) citam aqueles que estão relacionados à vida reprodutiva da mulher como: menarca precoce, primeira gestação com mais de 30 anos, uso de anticoncepcionais orais, menopausa tardia e utilização de terapia de reposição hormonal. No entanto, como fatores protetivos contra o câncer de mama pode-se destacar a multiparidade e a amamentação (Opdahl e cols. 2011; Anderson e cols. 2014).

Como fatores de risco pode-se citar aqueles que estão relacionados aos hábitos de vida das pacientes, como: tabagismo, consumo de álcool, sedentarismo, obesidade, entre outros (Ekelund e cols. 2015; Di Cello e cols. 2013; Peeters e cols. 2014). Acredita-se que as toxicidades de alguns desses fatores, como o tabaco e o álcool, em conjunto com a ação hormonal podem estar ligados ao início do processo

neoplásico (Rudel e cols. 2014; Di Cello e cols. 2013; Trichopoulos e cols. 2008). A obesidade também é considerada como um fator importante relacionado ao câncer de mama, pois trabalhos indicam que ela possui relação com os níveis aumentados de estrógeno circulante das pacientes (Rudel e cols. 2014; Di Cello e cols. 2013; Trichopoulos e cols. 2008).

Além desses fatores de risco, ainda existem os fatores ligados a hereditariedade, relacionados principalmente ao parentesco em primeiro grau, ou seja, mãe ou irmã apresentando risco aumentado para o desenvolvimento do câncer de mama (Berkey e cols. 2012; Goodwin e cols. 2015). Com isso, atualmente sabe-se que da totalidade de casos da doença, 5 a 10% deles estão ligados à história familiar das pacientes (Berkey e cols. 2012; Goodwin e cols. 2015).

O câncer de mama também possui fatores relacionados com mutações genéticas específicas, como as localizadas nos genes BRCA1 e BRCA2, que aumentam as chances de desenvolvimento da doença (Tao e cols. 2015; Goodwin e cols. 2015; Rebbeck e cols. 2015). As pacientes que apresentam esse tipo de mutação possuem risco elevado de desenvolvimento da doença, em cerca de 87% dos casos, segundo Tao e cols. (2015); Goodwin e cols. (2015) e Rebbeck e cols. (2015).

1.4. Critérios morfológicos do câncer de mama

1.4.1. Tipos histológicos

O câncer de mama possui classificações específicas para cada tipo de tumor que, por sua vez, tornam-se partes integrantes da oncologia moderna. Essas informações fornecem aos patologistas guias e recomendações que facilitam a

reprodutibilidade diagnóstica e prognóstica (Albrektsen e cols. 2012; Gobbi e cols. 2012). Nas últimas décadas, a publicação de documentos consenso como a Classificação de Tumores, da Organização Mundial da Saúde (OMS), tornou-se importante referência nas áreas da patologia e oncologia (IARC, 2012).

Nesta última edição (IARC, 2012), os tumores de mama estão classificados em: tumores epiteliais, lesões proliferativas intraductais e lesões precursoras, tumores epiteliais/mioepiteliais, lesões e neoplasias papilares, proliferações epiteliais benignas e tumores mesenquimais (IARC, 2012) (ANEXO I).

1.4.2. Grau

A utilização do grau histológico para classificar os tumores de mama tem sido empregada há muito tempo. Há relatos que sua introdução na patologia veio por meio de Von Hausemann em 1892 (Bloom e Richardson 1957; Freeman e cols. 1979). No entanto, o sistema de graduação veio a ser mais conhecido a partir do trabalho de Bloom e Richardson em 1957, que demonstraram a importância das características morfológicas das células neoplásicas relacionadas ao prognóstico da doença (Freeman e cols. 1979). Com o advento de novos métodos, a classificação de Bloom e Richardson foi modificada por Elston e Ellis para que houvesse uma melhor reprodutibilidade da graduação histológica (Sotiriou e cols. 2006).

Atualmente, o sistema adotado para verificar o grau dos tumores de mama é a classificação de Nottingham (NGS) - Bloom-Richardson modificado (Rakha e cols. 2012). O NGS tem como base avaliar o percentual de diferenciação tubular, pleomorfismo nuclear e índice mitótico, sendo importante no prognóstico da doença (Tsutsui e cols. 2003; Augusto e cols. 2006; Rakha e cols. 2012). De acordo com esse

índice, os tumores são classificados como: grau I (bem diferenciado), grau II (moderadamente diferenciado) ou grau III (pouco diferenciado – pior prognóstico) (Bloom e Richardson 1957; Press, 1990; Clark, 1994).

1.4.3. Estadiamento

1.4.3.1. Sistema TNM

O estadiamento tumoral é formado pelo sistema TNM (T: tamanho do tumor primário; N: estado linfonodal; M: metástases a distância), que é utilizado para determinar a extensão anatômica do tumor com base em critérios clínicos (cTNM) e patológicos (pTNM) (Benson 2003; Whitman e cols. 2006). O sistema TMN é preconizado pela *American Joint Committee on Cancer (AJCC)*, que o propõe como ferramenta para avaliar a doença, guiar o tratamento e fornecer informações a respeito do prognóstico (Coburn e cols. 2006; Veronesi e cols. 2006).

1.4.3.2. Tamanho tumoral

No sistema TNM, o “T” representa a descrição das graduações relacionadas ao tamanho tumor primário (Whitman e cols. 2006; Benson 2003). No câncer de mama classificado como Tx, o tumor primário não pode ser avaliado; o T0 não tem evidência de tumor primário; o Tis é um carcinoma *in situ*; no T1, as lesões que medem 2cm ou menos onde $T1 \geq 0,1\text{cm}$ possuem microinvasão e, as T1a, T1b, T1c apresentam respectivamente 0,1 a 0,5cm; 0,5 a 1cm e de 1 a 2 cm (Whitman e cols. 2006; Barros e cols. 2001; IARC, 2012). Tumores T2 podem medir entre 2 a 5 cm e, tumores T3 atingem dimensões maiores que 5 cm. Já, um tumor que possui qualquer tamanho,

porém com invasão epitelial, é considerado T4 e pode ser subdividido em: T4a (invasão torácica), T4b (neoplasias com ulcerações da pele, edema e nódulos), T4c (T4a + T4b) e T4d (carcinomas inflamatórios) (Whitman e cols. 2006; Coburn e cols. 2006; IARC, 2012).

1.4.3.3. Estado linfonodal

O estado linfonodal (N) também possui uma graduação específica, sendo ele de grande importância para o planejamento do tratamento adequado e o fornecimento do prognóstico relacionado à doença (Whitman e cols. 2006). Dessa forma, Nx são linfonodos que não podem ser avaliados e, pN0 é indicio de que não há metástases linfonodais (Barros e cols. 2001; Benson 2003; IARC, 2012).

Quando um linfonodo lateral móvel é acometido por metástases ele é classificado como N1 (Whitman e cols. 2006; IARC, 2012). Linfonodos com processo metastático são N2, os quais podem ser ipsilaterais fixos (N2a), gânglios linfáticos internos (N2b) (Whitman e cols. 2006; IARC, 2012). Invasão clavicular decorrente de metástases linfáticas ou invasão clinicamente evidente ao longo dos linfonodos axilares são N3, subdivididos em: N3a, gânglios linfáticos infraclaviculares e linfonodos axilares; N3b, nódulos mamários internos e linfonodos axilares; e, por fim, N3c, metástases em linfonodos supraclaviculares. (Whitman e cols. 2006; IARC, 2012).

1.4.3.4. Metástases a distância

O sistema TNM também avalia a presença de metástases a distância, que é subdividido em Mx, M0 e M1 (Veronesi e cols. 2006; IARC, 2012). Quando não faz-se possível avaliar as metástases a distância preconiza-se Mx e M0, quando não há evidências de doença metastática a distância (Veronesi e cols. 2006; IARC, 2012). Se forem encontradas metástases a distância ocasionadas pelo câncer de mama, utiliza-se M1 (Veronesi e cols. 2006; IARC, 2012).

1.4.4. Imunohistoquímica

A imunohistoquímica tem sido uma técnica indispensável para auxiliar a patologia durante décadas. Atualmente, os principais marcadores estabelecidos pela literatura são: os receptores hormonais (estrógeno e progesterona), superexpressão do receptor 2 do fator de crescimento epidérmico humano (HER-2), citoqueratina 5/6 (Ck 5/6), Ki-67 e EGFR. Esses marcadores, junto com as variáveis clinicopatológicas do câncer de mama, são comumente utilizados para classificar o fenótipo molecular do câncer de mama (Lin e Chen 2014; Vallejos e cols. 2010).

1.4.4.1. Fenótipos moleculares

De acordo com as características imunohistoquímicas, o câncer de mama pode ser subdividido em 6 principais fenótipos: luminal A, luminal B, luminal B (luminal HER-2), superexpressão de HER-2, triplo-negativo basaloide e triplo-negativo não basaloide (Tab. 1) (Cirqueira e cols, 2011; Anderson e cols. 2014).

Perfis de imunomarcção para composição do painel imunohistoquímico e classificação de tumores de mama.

Subtipos	Padrão de imunomarcção
Luminal A	RE+ e/ou RP+, HER2- e KI-67 $\leq$ 14%
Luminal B	RE+ e/ou RP+, HER2- e Ki-67 $\geq$ 14%
Luminal B (Luminal HER-2)	RE+ e/ou RP+, HER2+ (Luminal HER2)
Superexpressão de HER-2	RE- e/ou RP-, HER2+
Triplo-negativo basaloide	RE-, RP-, HER2-, Ck5/6+ e/ou EGFR+
Triplo-negativo não basaloide	RE-, RP-, HER2-, Ck5/6- e/ou EGFR-

RE+: Receptor de estrógeno positivo; RE-: Receptor de estrógeno negativo; RP+: Receptor de progesterona positivo; RP-: Receptor de progesterona negativo; HER-2-: Sem superexpressão do receptor 2 do fator de crescimento epidérmico humano (HER-2); HER-2+: Superexpressão do receptor 2 do fator de crescimento epidérmico humano (HER-2); Ki-67: Proteína Ki-67; Ck5/6+: Citoqueratina 5/6 positiva; Ck5/6-: Citoqueratina 5/6 negativa; EGFR+: Receptor do fator de crescimento epidérmico positivo; EGFR-: Receptor do fator de crescimento epidérmico negativo.

Fonte: Adaptado de Cirqueira e cols. (2011) e Anderson e cols. (2014)

O fenótipo Luminal A é o mais frequente de todos os cânceres de mama, 50% a 60% dos casos (Yersal e Barutca 2014; Sun e cols. 2014). Eles são caracterizados por apresentarem níveis mais altos de RE e baixa expressão dos genes relacionados à proliferação celular (KI-67 $\leq$ 14%) (Yersal e Barutca 2014; Sun e cols. 2014). Frequentemente, possuem baixo grau histológico, pouco pleomorfismo nuclear, atividade mitótica reduzida e, ainda, tipos histológicos de bom prognóstico (Yersal e Barutca 2014; Sun e cols. 2014).

O Luminal B compreende 15% a 20% dos cânceres de mama (Yersal e Barutca 2014; Sun e cols. 2014). Esse fenótipo é mais agressivo, pois possui maior grau histológico e alto índice proliferativo ($KI-67 \geq 14\%$). (Yersal e Barutca 2014; Sun e cols. 2014). Suas características propiciam um pior prognóstico, influenciando no aumento da taxa de recidiva e na menor sobrevida das pacientes, quando comparado ao Luminal A (Yersal e Barutca 2014; Sun e cols. 2014).

Os cânceres de mama do tipo HER-2 possuem esse nome por apresentarem a superexpressão da proteína HER-2, codificada pelo protooncogene *HER-2* (Yersal e Barutca 2014). São 15% a 20% dos fenótipos encontrados e, entre todos, são os que possuem comportamento clínico mais agressivo (Yersal e Barutca 2014). Os tumores caracterizados pela elevada expressão de HER-2, em 75% dos casos, possuem grau histológico elevado com mutações do *p53* e, apenas 40% apresentam positividade para expressão dos receptores hormonais (RE e RP) (Vuong e cols. 2014; Yersal e Barutca 2014).

Cânceres que não superexpressam a HER-2 e são negativos para RE e RP são chamados triplo-negativos (Vuong e cols. 2014; Yersal e Barutca 2014). São aproximadamente 10% dos cânceres de mama, e podem ser divididos em basaloides e não basaloides (Cirqueira e cols. 2011; Anderson e cols. 2014). Os tumores basaloides são os mais comuns e representam 8% a 37% dos cânceres de mama. A maioria são *ductais infiltrantes* com padrão de crescimento sólido, comportamento clínico agressivo e elevada taxa de progressão tumoral, pois, de maneira geral, são associados ao alto grau histológico (Yersal e Barutca 2014). (Yersal e Barutca 2014).

A identificação e diferenciação dos fenótipos moleculares no câncer de mama, através da análise do perfil imunohistoquímico, possui importância em relação ao prognóstico da doença. A expressão desses marcadores está diretamente relacionada

ao tratamento adequado a cada tipo de câncer, fazendo com que essas características tenham sido amplamente estudadas nas últimas décadas (Cheng e cols. 2013; Chen e cols. 2014).

1.5. Marcadores Tumorais

Os marcadores tumorais são componentes teciduais, plasmáticos e genéticos capazes de definir diferentes características a uma patologia (Sato e cols. 2014). Através do estudo dos marcadores tumorais consegue-se ter uma melhor compreensão a respeito das bases moleculares e celulares de iniciação e progressão do câncer (Gobbi 2012).

A avaliação rotineira dos marcadores tumorais é indicada em todos os casos de câncer de mama por comitês de consenso oncológicos internacionais (Gobbi 2012). Estudos mostram que a identificação de RE, RP, HER-2, Ck 5/6, Ki-67 e EGFR possuem fator prognóstico e preditivo relativos à doença (Gilcrease 2013; Brufsky e cols. 2014; O-charoenrat 2015). Em contrapartida, cânceres negativos, para os marcadores tumorais, conferem às pacientes um pior prognóstico por apresentarem características clínicas ligadas ao maior tamanho tumoral, estadiamento, grau elevado e alto risco de desenvolvimento de metástases a distância (Gilcrease 2013; Brufsky e cols. 2014; O-charoenrat 2015).

Com o advento dos avanços tecnológicos, incluindo metodologias e surgimento de novos anticorpos, o estudo dos biomarcadores *ceramida galactosiltransferase* (*UGT8*) e *GATA3* possui grande importância para entendimento dos mecanismos relacionados ao câncer de mama (Dziecedil Giel e cols. 2010; Hattori e cols. 2015)

1.5.1. UGT8

O *ceramide galactosyltransferase* (*UGT8*) é um gene que codifica a galactosylceramida (GalCer), uma proteína sintetizada no retículo endoplasmático das células normais no organismo (Erez-Roman e cols. 2010). A expressão celular de *UGT8* encontra-se ligada de forma direta à quantidade de GalCer sintetizada por ele, ou seja, quanto maior a quantidade de GalCer, mais expresso estará o *UGT8* (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015).

A GalCer tem sua síntese através da transferência de galactose para ceramida, a qual possui a função de molécula mensageira envolvida em mecanismos básicos de processos celulares como a interrupção do crescimento celular, diferenciação, senescência e apoptose (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015). A ceramida está presente na construção dos complexos esfingolipídicos, componentes estruturais importantes da membrana plasmática, que também atuam como molécula de sinalização secundária ao estresse celular (Woodacre e cols. 2013; Senkal e cols. 2010).

Através da síntese de GalCer, a ceramida serve como mediadora do processo de morte celular controlado por cascatas relacionadas à apoptose como aquelas responsáveis pela ativação das caspases (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015). Existem outras moléculas ligadas à ceramida, como a ceramida fosforilada, precursora da ceramida dihydrosphingosine (DHSP) e fitoesfingosina (PHSP), que agem de forma antagônica a GalCer, onde são responsáveis pelos sinais das vias promotoras da proliferação celular (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015). Uma vez que a baixa expressão de *UGT8* resulta na baixa síntese de GalCer e, por consequência, na baixa ceramida, este marcador também

acaba relacionando-se com as vias promotoras da proliferação celular, pois estas não impulsionam a morte celular (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015).

Embora a resposta celular à ceramida seja importante para regulação da proliferação celular e das vias responsáveis pela morte das células, os mecanismos moleculares mais precisos para essa regulação continuam exigindo atenção (Woodacree cols. 2013, Senkal e cols. 2015). É importante que os estudos desses mecanismos possam auxiliar na compreensão da resposta celular ao estresse a fim de buscar estratégias para modifica-las (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015). Essas estratégias podem ser relacionadas à aceleração do processo de morte celular ou evitar danos causados por acúmulos intracelulares anormais de substâncias nocivas ao metabolismo da célula (Rao e cols. 2007, Woodacree cols. 2013, Senkal e cols. 2015).

No câncer de mama, o *UGT8* e a GalCer têm seus estudos ligados ao processo metastático e ao prognóstico da doença, pois encontram-se superexpressos em pacientes com doença metastática pulmonar devido à existência de um câncer de mama (Choi e cols. 2012; Dziecedil Giel e cols. 2010). Neste contexto, Dziecedil Giel e cols. (2010) propuseram que cânceres de mama RE negativos (pior prognóstico) combinados com a inibição de GalCer – em consequência inibição da apoptose – aumentam as chances de desenvolvimento de metástases a distância no organismo (Dziecedil Giel e cols. 2010).

O *UGT8* também possui ligação com o grau tumoral, pois sua expressão como gene está relacionada à diferenciação celular, principalmente nos sistemas nervoso central e periférico (Senkal e cols. 2015, Choi e cols. 2012; Dziecedil Giel e cols. 2010). Estudos relacionados a essa propriedade do *UGT8* relataram que a sua baixa

expressão no câncer de mama está correlacionada a células tumorais pouco diferenciadas, ou seja, ao câncer de mama com grau mais elevado (Choi e cols. 2012; Dziecedil Giel e cols. 2010). O estudo de Dziecedil Giel e cols. (2010) também demonstrou que a alta expressão do marcador *UGT8* no câncer de mama com grau mais baixo propicia, a este grupo de pacientes, uma maior sobrevida relacionada à doença (Dziecedil Giel e cols. 2010).

Sabe-se que expressão de *UGT8* e GalCer no câncer de mama está ligada ao processo de morte celular, porém os mecanismos relacionados às vias apoptóticas e ao processo metastático ainda apresentam lacunas e devem continuar em estudo.

1.5.2. GATA3

O *GATA3* é um membro pertencente a uma família de 6 fatores de transcrição dedo de zinco (*GATA1-6*), os quais se ligam ao consenso A/ TGATAA /G e estão situados no cromossomo 10, na localização 10p15 e possuindo 10 exons (Ciocca e cols. 2009, Takaku e cols. 2015). A maioria das mutações ligadas à família *GATA* estão localizadas no exon 1 e na primeira região do exon 5 (Ciocca e cols. 2009, Takaku e cols. 2015). Por sua vez, o *GATA3* codifica a GATA-proteína de ligação 3 (*GATA-3*), pertencente à família dedo de zinco, ligada a diversos processos de diferenciação celular (Jiang e cols. 2014).

De maneira geral, a família *GATA* está envolvida nos processos de eritropoiese mielóide (*GATA 1,2 e 3*), desenvolvimento cardíaco e intestinal (Shan e cols. 2014, Takaku e cols. 2015). A expressão do *GATA3* também está relacionada à identidade celular, pois possui associação com a especificação dos tipos de células do sistema imunológico em que a ação é integrante da diferenciação de outras linhagens

celulares no organismo (Ciocca e cols. 2009, Shan e cols. 2014, Takaku e cols. 2015). Além disso, o *GATA* faz parte da especificação de células que incluem neurônios do sistema nervoso simpático, células T-helper, bainha radicular interna de folículos pilosos, células adiposas, ductos nefróticos, cóclea do ouvido e células ductais mamárias (Takaku e cols. 2015, Yan e cols. 2015). Na glândula mamária, ele se encontra com maior expressão nas células epiteliais luminais, que revestem as estruturas ductais da mama, estabelecendo a manutenção da identidade das células luminais na mama (Shan e cols. 2014, Takaku e cols. 2015).

No câncer de mama, o *GATA3*, além de estar associado ao epitélio luminal, possui importância na análise genômica da doença, pois estudos têm demonstrado recentemente que esse gene é um alvo frequente de mutações relacionadas ao câncer de mama (Calvo e cols. 2013; Shan e cols. 2014). Essas mutações são identificadas em cerca de 10% dos tumores de mama e a maioria delas estavam limitadas a um único alelo e, a expressão de ambos os alelos (selvagem e mutado) encontraram-se de forma quase que equivalente (Ciocca e cols. 2009, Takaku e cols. 2015). De acordo com os mesmos estudos, a perda da expressão do *GATA3* está ligada a cânceres com propensão ao crescimento invasivo e desenvolvimento de metástases em outros sítios, ressaltando a importância do estudo da expressão desse biomarcador no câncer de mama (Ciocca e cols. 2009, Takaku e cols. 2015).

O mau prognóstico ligado ao *GATA3*, no câncer de mama, está relacionado à expressão de RE e às mutações encontradas no gene (Krings e cols. 2014). A alta expressão de *GATA3* correlaciona-se de forma significativa com expressão dos receptores de estrógeno, fazendo com que atuem juntos como coativadores do câncer de mama, pois um estimula a expressão do outro (Takaku e cols. 2015; Gulbahce e cols. 2013; Albergaria e cols. 2009). Além disso, a relação existente entre tumores RE

positivos com a expressão de *GATA3* demonstram que as mudanças do perfis dos cânceres de mama fornecem novos fenótipos ligados a um pior prognóstico para as pacientes com a doença (Albergaria e cols. 2009; Hattori e cols. 2015). Thakkar e cols. (2015) publicaram, em um estudo recente com 109 amostras RE positivas, que todas apresentavam expressão alta de *GATA3* (Thakkar e cols. 2015). Esse índice de marcação faz com que o *GATA3* venha a ser estudado intensivamente como potencial imunomarcador relacionado às vias dos receptores hormonais no câncer de mama, pois está ligado ao estímulo de estradiol, interferindo no progresso do ciclo celular tumoral (Thakkar e cols. 2015).

Com o advento de estudos genômicos no câncer de mama, hoje se tem conhecimento da frequência de mutações do *GATA3* na influência do direcionamento tumoral (Ciocca e cols. 2009, Takaku e cols. 2015). Estudos mostraram que as mutações do *GATA3*, frequentemente relacionadas ao câncer de mama, estão localizadas nos exons 5 e 6, no entanto, salienta-se que o espectro de mutações nesse gene é ainda de difícil identificação, tanto para ganho de funções, como para perda das mesmas (Ciocca e cols. 2009, Takaku e cols. 2015, Yan e cols. 2015). Acredita-se que a regulação dos níveis de proteína GATA-3, codificada pelo *GATA3*, seja essencial para manutenção fisiológica das células epiteliais luminais e, as mutações que resultam na truncagem prematura dessa proteína promovem a perda da mesma função nas células luminais mamárias. Como o *GATA3* está relacionado ao estradiol, os altos níveis desse hormônio manifestam-se na transcrição dos genes em regiões reguladas pelas funções dos REs (Takaku e cols. 2015, Yan e cols. 2015, Jiang e cols. 2014).

A regulação do nível proteico de GATA-3 pode ser uma das alternativas de estabilidade relacionada às mutações do gene *GATA3* (Takaku e cols. 2015, Yan e

cols. 2015, Jiang e cols. 2014). Um exemplo deste modelo está presente no câncer de próstata, cujos baixos níveis de GATA-3 em tumores proteíno-deficientes podem acabar conduzindo à progressão tumoral e ao processo metastático, pois são influenciados pela produção hormonal (Takaku e cols. 2015, Li e cols. 2014)

Nos últimos anos, o *GATA3* emergiu como um fator importante na função da glândula mamária normal e no câncer de mama. A descoberta das mutações da GATA-3, anteriormente não caracterizadas, podem fornecer respostas a questões ainda não respondidas sobre o câncer de mama. Embora o estudo genômico tenha elucidado muitas dessas questões, ainda há trabalho relacionado ao impacto das mutações no câncer de mama.

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2. Objetivo Principal

Identificar a expressão dos marcadores *UGT8* e *GATA3* em amostras de pacientes com tumor primário de mama;

2.1. Objetivos específicos

- Análise do perfil demográfico das pacientes;
- Associar e correlacionar a expressão de *UGT8* e *GATA3* com:
 - Perfil imunohistoquímico;
 - Tamanho e grau tumoral;
 - TMN;
 - Subtipos moleculares;

3. Artigo 1

O artigo intitulado “**ASSOCIATION OF THE EXPRESSION OF THE MARKERS *UGT8* AND *GATA3* IN BREAST CANCER**” encontra-se redigido em inglês, segundo as normas da revista The Breast.

**ASSOCIATION OF THE EXPRESSION OF THE MARKERS *UGT8* AND *GATA3* IN
BREAST CANCER**

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ABBREVIATIONS

TNM – Primary tumor, nodal status and distant metastasis.

G – Tumor grade.

ER – Estrogen receptor.

PR – Progesterone receptor.

HER-2 – Human Epidermal growth factor Receptor 2.

Ck 5/6 – Cytokeratin 5/6

Ki-67 – Ki-67 protein

p53 – p53 gene

UGT8 – Ceramide galactosyltransferase.

GalCer – Galactosylceramide.

mRNA – Messenger Ribonucleic Acid

GATA3 – Gene encoding the GATA-3 protein

GATA-3 – GATA binding protein 3

HE – Hematoxylin and Eosin

ABSTRACT

INTRODUCTION: Breast cancer is the most common neoplasia among women worldwide. It has different molecular phenotypes related to the expression of the tumor markers. Besides the markers already established by literature, we can mention other important markers related to breast cancer, like the *ceramide galactosyltransferase* (*UGT8*) and the *GATA3*. The study of these markers aims to make possible a better understanding of the disease.

OBJECTIVE: Associate the expression of *UGT8* and *GATA3* with the size and the TNM cancer staging and the molecular phenotypes in samples of patients with primary breast tumor.

MATERIALS AND METHODS: For this research, patients with breast cancer in the mastology clinic from the referred hospital were selected. They answered a questionnaire and consented in the use of their anatomopathological reports for research purposes. After that, the immunohistochemistry of the markers *UGT8* and *GATA3* was made in the samples and, the result, was associated with TNM, staging, grade and molecular subtypes.

RESULTS: 174 questionnaires provided by patients with questions referring to demographic data, reproductive life and risk factors and 121 anatomopathological reports were included in this research. Concerning the marks in this study, significant associations between elevated expression of *UGT8* with the tumor size of 2cm ($P=0.043$) and negative expression with the PR ($P=0.029$) were found. The high expression of *GATA3* was associated with stage I tumors ($P=0.05$) and its absence of expression was significant in stage III tumors ($P=0.05$) with sizes between 2.1cm to 5cm ($P=0.019$).

CONCLUSION: Based on the found results, we can conclude that there was a significant association between the expression of the potential prognostic markers, *UGT8* and *GATA3*, and the size of the tumors, expression of PR and staging of the studied samples.

Keywords: TNM, *UGT8*, *GATA3*, Breast cancer.

INTRODUCTION

Breast cancer is a disease with heterogeneous features, what is reflected in its molecular classification, morphology, clinical course and response to treatment [1]. It is one of the most common neoplasias in the female population, since, in 2012, it had a worldwide incidence of 1.6 million new cases [2]. In Brazil, the 2015 estimate was 57.120 cases, with mortality rate of 14.207 women [3].

The disease has different phenotypes and the study of its immunohistochemical profile is very important in its classification. The molecular phenotypes are defined by the analysis of the immunohistochemical expression of estrogen and progesterone receptors (ER and PR), HER-2, cytokeratin 5/6 (Ck 5/6), Ki-67 and *p53* [4][5][6]. Besides the markers already established by literature, other important markers related to breast cancer can be mentioned, like the *ceramide galactosyltransferase* (*UGT8*) and the *GATA3* [7][8]. These new markers serve to provide a better understanding of the mechanisms related to the development of breast cancer [5][9][10].

The *UGT8* is a gene which codifies the galactosylceramide (GalCer), a synthesized protein of the endoplasmic reticulum [11]. The synthesis of the GalCer happens through the transference of galactose into ceramide, which has the function of messenger molecule involved in basic mechanisms of cellular processes such as the interruption of cell growth, differentiation, senescence and apoptosis [12][13][14]. Through the synthesis of GalCer, the ceramide has the function of mediator of the process of cell death controlled by cascades related to apoptosis, such as the ones responsible for the activation of caspases [12][13][14]. The cellular expression of *UGT8* is related to the intracellular GalCer, because the higher is the amount of GalCer, the greater will be the expression of *UGT8* [12][13][14].

The *GATA3* belongs to a family of 6 zinc finger transcription factors (*GATA1-6*), which are connected to the consensus *A/ TGATAA /G* and are located in the chromosome 10, in the location 10p15, having 10 exons [15][16]. This transcription factor codifies the GATA binding protein 3 (*GATA-3*), belongs to the zinc finger family, related to several processes of cellular differentiation, such as: specification of neural cells of the sympathetic nervous system, T-helper cells, inner root sheath of hair follicles, adipocytes, nephrotic ducts, ear cochlea and mammary ductal cells (luminal) [16][17]. In breast cancer, the loss of *GATA3* expression is related to cancers with propensity of invasive growth and development of distant metastasis, highlighting the importance of research on this marker related to metastatic process [15][16].

Thus, the aim of this study is to associate the expression of *UGT8* and *GATA3* in the samples of primary breast cancer patients with the TNM, tumor grade, immunohistochemical profile and molecular phenotypes.

MATERIALS AND METHODS

Patients and ethical considerations

A transversal study was performed between March, 2014 and December, 2015. The inclusion criteria of this research were: Female patients with previous diagnostic of breast cancer, older than 18 years old and accompanied by the mastology team from the clinic of *Sistema Único de Saúde* (SUS) from Santa Rita Hospital, in the Santa Casa de Misericórdia de Porto Alegre Hospital Complex.

The patients were included in the research after the signature of a Term of Consent in the moment of the interview. The present study was approved by Santa

Casa de Misericórdia de Porto Alegre Hospital Complex Ethics Committee, under ruling number 525.170, meeting the specifications of the Resolution 466/2015 of CNS/MS, Brazil and Declaration of Helsinki, 2013.

Questionnaires

The population in this study was described through a valid questionnaire (Ferreira et al. 2015), answered by patients of the mastology clinic. The questionnaire was composed of questions about demographic data, reproductive life of the patients, risk factors, diagnosis and treatment.

Samples

In order to do this research, anatomopathological reports from patients with breast cancer were selected and included in the study. These reports were first in the pathology section from the hospital.

Immunohistochemistry

The immunohistochemical profile of primary breast tumors was established according to the histopathological reports provided by the hospital. Information about staging, tumor's size, grade and molecular phenotype were also extracted from these same reports.

The slides were revised through optical microscopy in order to confirm the cell representativeness. The immunohistochemical technique performed here was based

on the methodology of Dziecedil Giel et al. (2010), Gonzalez et al. (2013) and Liu et al. (2014), using *UGT8* (dilution 1:150) and *GATA3* (dilution 1:800), both of them from Abcam® - Cambridge, United Kingdom, with positive and negative controls of human brain and breast, respectively (Tab. 1).

Sample analysis

The immunohistochemical analysis was independently made by two analysts by optical microscopy and 10% of the final results were revised by a third pathologist. In both cases, antibodies had a positive mark and brown coloration, but in different sites: cytoplasmic mark to *UGT8* and nuclear mark to *GATA3* (Fig. 1). First, each sample was analyzed according to their color intensity in three different fields ("Hot Spots"), where they were categorized, based on the methodology of Dziecedil Giel et al. (2010) and Cimino-Mathews et al. (2013), in: 0 (negative), + (positive weak), ++ (positive moderate) and +++ (positive strong) (Fig. 1 e Fig. 2).

Statistical analysis

The obtained data were tabulated in Microsoft Office Excel 2007 and the statistical analysis was made in the software Statistical Package for the Social Sciences (SPSS 21® - Chicago, Estados Unidos).

The results were presented using absolute and relative frequencies (count and percentage). Mean and standard deviation of the quantitative variables were also calculated, and, concerning the categorical variables, the Chi-Square Test of Independence was calculated, assigning the significance level of 5% ($P=0,05$).

RESULTS

Description of the population of the study

This study is composed of 174 patients who signed the Term of Consent and answered the questionnaire. The description of the population of the study appears on **Table 2**. According to the obtained answers, it was observed that the average age of breast cancer patients is 57 years old ($SD \pm 14$). The patients are white, had their menarche between the ages of 12 and 14 years, had children, breastfed their children, made use of hormonal contraceptive medications, entered menopause between the ages of 41 and 55 years, and present BMI between 25 and 29.9 (World Health Organization – WHO). They also do not practice any physical exercises and do not have smoking or drinking habits. The interviewed women did not reported family history of breast cancer, but they mentioned family history of other types of cancer, especially first grade ones.

Association of the expression of UGT8 and GATA3

The results obtained from the association of the expression of the studied markers when related to TNM, grade, immunohistochemical profile and molecular phenotypes of the breast tumors in analysis are described on **Tables 3 and 4**.

Concerning the positivity of markers *UGT8* and *GATA3*, it was observed that they both had a high level of expression (strong: 3+) in the studied breast tumors (47.1% and 52%, respectively).

In relation to the marker *UGT8*, it is possible to observe that there was a significant association between the high level of expression of *UGT8* and the tumor size of 2cm ($P=0.043$). Likewise, the negative and the weak positive expressions (1+) ($P=0.043$) were associated with tumor sizes from 2.1cm to 5cm. There was also a significant association between the absence of expression of progesterone receptors (PR) in the tumor samples and the absence of marking of *UGT8* in the same studied samples of breast cancer ($P=0.029$).

The high level of expression (3+) of the marker *GATA3* is significantly associated with the stage I tumors ($P=0.05$) and the negative expression of the same marker is associated with stage III tumors ($P=0.05$). The absence of expression of *GATA3* was also observed, associated with tumors with size between 2.1cm and 5cm ($P=0.019$).

DISCUSSION

This research had as main focus the association between the immunohistochemical expression of the markers *UGT8* and *GATA3* and the variables related to size, lymph node status, distant metastasis, grade, immunohistochemical profile and molecular subtypes of breast tumors. The study also generated demographic data about the patients, such as: age, reproductive life, habits and hereditary factors. These data were important to establish the profile of the patients in study, along with the prognostic and predictive factors related to tumor markers [10].

From the results obtained through the association between the breast tumor characteristics and the expression of the analyzed markers, a significant association between 2cm tumors and a high level of expression of *UGT8* was observed. It was

also observed an association between 2.1 to 5cm tumors and negative and low expression (weak positive) of *UGT8*.

There was a significant association between the negative expression of *UGT8* and the negative PR ($P=0.029$). Other researches in this field treated about the existence of a greater association between ER-negative tumors and the *UGT8* [7][9][20]. Although the work of Dziecedil Giel et al. (2010) relates negativity of ER tumors to the increasing of *UGT8* in malignant cells of primary breast carcinoma, a significant association between PR-negative breast tumors and *UGT8* was found. Owczarek et al. (2013), however, proposed that the *UGT8* has a significant index related to negative hormonal receptors (ER and PR) and to tumor aggressiveness, assigning the ceramide the role of potential marker in a prognostic evaluation of metastasis in breast neoplasias when connected to these negative tumors [9].

Concerning the *GATA3*, it was found that the most notable levels of expression of this marker appeared in the significant associations between stage III tumors and negative expression, and between stage I tumors and high level of expression (strong positive). In tumors of 2.1cm to 5cm there were also significant results, but when associated with the negative expression of *GATA3*. Some studies suggest that the low level of expression of *GATA3*, when associated with histological features like growth of tumor mass, lymph node involvement, grade and molecular phenotype, is connected to a lower survival rate for the patients [10][21][22].

There was no significant association between none of the markers belonging to the immunohistochemical panel of breast cancer or molecular phenotype. The research presented by Gulbahce et al. (2013) shows a positive correlation between the marker *GATA3* and the positive expression of ER in 97% of the cases studied (n=541) and, similarly to the results obtained here, the research did not find a

significant correlation between molecular phenotype, lymph node involvement and PR [23]. The positive correlations of *GATA3* are elucidated by the work of Cohen et al. (2014), in which the expression of ER along with the overexpression can act like tumor promoters, especially in luminal molecular subtypes, stimulating the progression of the disease [24]. It can be noticed, however, a low quantity of reports in literature exploring this high level of expression of *GATA3* in negative breast tumors when related to hormonal receptors [8][18].

It was understood that this study presented statistical limitations since, due to the low sample size, accompanied by losses, more significant associations between the other variables of the study could not be obtained.

CONCLUSION

Based on the found results, we can conclude that there was a significant association between the expression of *UGT8* and *GATA3* and tumor size, expression of PR and primary breast cancer staging.

It is suggested that new researches with these potential markers should be performed and that their association with apoptosis pathways will be important for a better understanding of the progression of the disease.

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Tabela 1: Description of the antibodies used for immunohistochemistry in breast primary tumor: UGT8 and GATA3.

Antibody	Mark	Códe	Description	Reactive species	Positive control	Dilution
Anti-UGT8	Abcam	ab 170351	Polyclonal rabbit for UGT8	Rats and Humans (paraffin embedded samples)	Human brain and rats cerebellum	1:150
Anti-GATA3	Abcam	ab 106625	Polyclonal rabbit for GATA3	Rats, rabbit and Humans (paraffin embedded samples)	Human brain and rats cerebellum	1:800

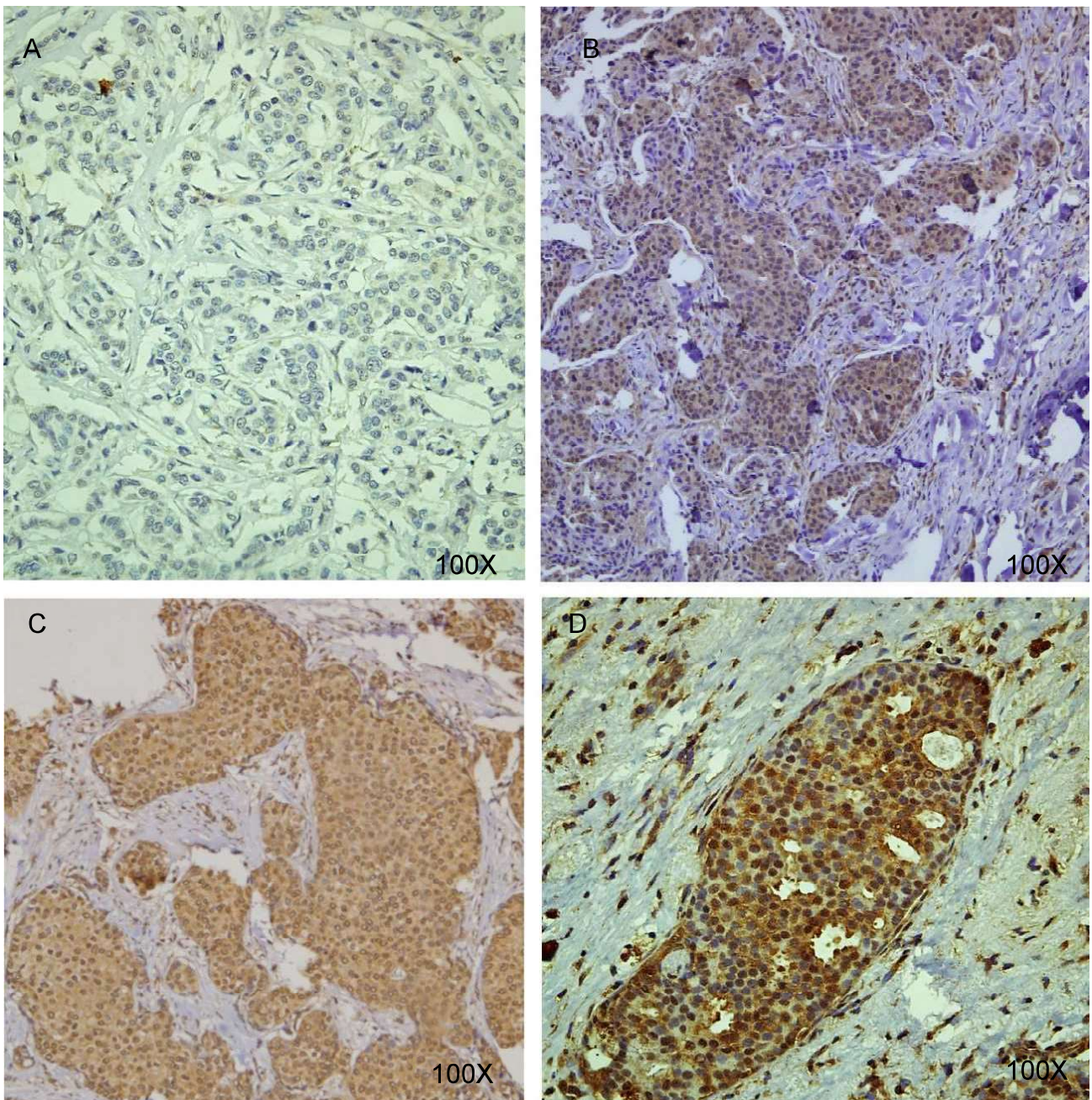


Figura 1: Expression of marker UGT8 in Breast Infiltrating Ductal Carcinoma. Objective 100X. A: 0 (negative), B: + (weak positive), C: ++ (moderate positive) e D: +++ (strong positive).

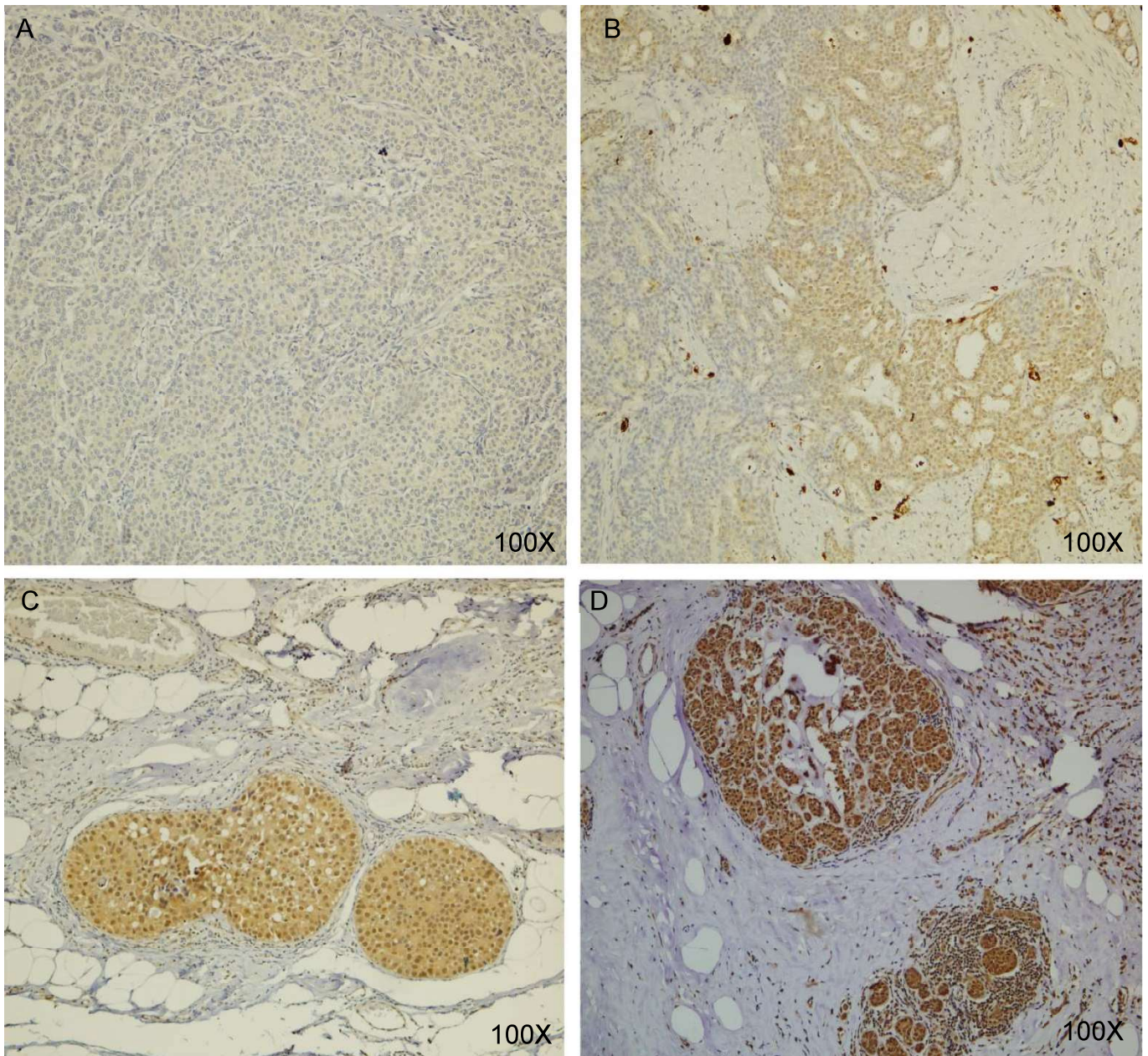


Figura 2: Expression of marker GATA3 in Breast Infiltrating Ductal Carcinoma. Objective 100X. A: 0 (negative), B: + (weak positive), C: ++ (moderate positive) e D: +++ (strong positive).

Tabela 2: Previous history data of patients with breast cancer.

Variable	N = 174**	%	Variável	N = 174**	%
Mean and standard deviation of age at diagnosis (years)	57 (DP ± 14)				
Menarche(years)			Current Weight/height - BMI		
<12	43	24,7	25 - 29,9	57	35,6
12 - 14	95	54,6	≥ 30	55	34,4
≥15	28	16,1		48	30
Self-declared race			Weight/height - BMI before diagnosis		
White	143	80,7	18,6 - 24,9	57	36,1
Black	8	4,8	25 - 29,9	55	34,8
Brown	1	*	≥ 30	46	29,1
Parity	148	89,2	Pratice of physical exercise	50	29,1
			Smoking habit	71	41
Mean and standard deviation of children per woman	3 (DP ± 2)				
Breastfeed	141	89,8	Time smoking habit (years)		
			≤1	1	1,4
2 - 4			2 - 4	5	7
≥5			≥5	9	12,7
Hormonal anticonceptive use	129	75,9	Alcohol intake	31	18,1
Time for hormonal anticonceptive use (years)			History of chronic disease	100	60,2
≤5	29	22	Family history of breast cancer	52	29,9
6 - 10	25	18,9			
≥11	75	56,8	Degree of kinship		
Menopause	145	84,3	1° grade	20	38,5
			2° grade	21	40,4
Menopause age (years)			3° grade	11	21,2
≤40	13	9	Family history of other type of cancer	69	44,2
41 - 55	121	84			
≥56	8	5,6	Degree of kinship		
Hormone replacement	24	16,4	1° grade	49	64,5
			2° grade	18	23,7
Time for hormone replacent (years)			3° grade	9	11,8
≤1	13	50			
2 - 4	3	11,5			
≥5	4	15,4			

*Values ≤1

** Data not reported in the variables and treated as "unknown" by the interviewed patients.

Tabela 3: Expression associated of marker UGT8 with TNM, immunohistochemistry profile and molecular phenotype of breast cancer.

Variable	UGT8 (% expression associated)					P
	N (%)	0 (Negative)	+	++ (Weak)	+++ (Strong)	
Staging		23 (19,1)	6 (5)	35 (29,1)	57 (47,5)	0,234
I	30 (26,7)	2 (8,7)	*	12 (34,3)	18 (31,6)	
II	50 (41,7)	11 (47,8)	4 (8,0)	13 (37,1)	22 (38,6)	
III	34 (28,3)	10 (43,5%)	1 (2,0)	8 (22,9)	15 (26,3)	
IV	4 (3,3)	*	*	2 (5,7)	2 (3,3)	
Tumor size (cm)		3 (13)	*	11 (31,4)	22 (38,6)	0,043
≤2,0	36 (30)					
2,1 - 5	69 (57,5)	19 (82,6)	5 (100)	18 (51,4)	27 (47,4)	
≥5	15 (12,5)	1 (4,3)	*	6 (17,1)	8 (14)	
Distant metastasis		23 (100)	5 (100)	33 (94,3)	55 (96,5)	0,622
No	116 (96,7)	*	*	2 (5,7)	2 (3,5)	
Yes	4 (3,3)					
Nodal status		12 (52,2)	4 (80)	22 (62,9)	39 (68,4)	0,718
0	77 (64,2)	5 (21,7)	*	8 (22,9)	11 (19,3)	
1	24 (20)	4 (17,4)	*	3 (8,6)	4 (7)	
2	11 (9,2)	2 (8,7)	1 (20)	2 (5,7)	3 (5,3)	
3	8 (6,7)					
Grade		2 (8,7)	*	7 (20)	7 (12,3)	0,606
I	16 (13,3)	11 (47,8)	2 (40)	12 (34,3)	29 (50,9)	
II	54 (45)	10 (43,5)	3 (60)	16 (45,7)	21 (36,8)	
III	50 (41,7)					
RE status		15 (65,2)	3 (50)	30 (88,2)	41 (71,9)	0,092
Positive	89 (74,2)	8 (34,8)	3 (50)	4 (11,8)	16 (28,1)	
Negative	31 (25,8)					
RP status		9 (39,1)	3 (50)	25 (73,5)	40 (70,2)	0,029
Positive	77 (64,2)	14 (60,9)	3 (50)	9 (26,5)	17 (29,8)	
Negative	43 (35,8)					
HER-2 status		5 (21,7)	2 (33,3)	7 (20,6)	7 (12,3)	0,453
Positive	21 (17,5)	18 (78,3)	4 (66,7)	27 (79,4)	50 (87,7)	
Negative	99 (82,5)					
Molecular phenotype		14 (60,9)	2 (33,3)	25 (71,4)	41 (71,9)	0,47
Luminal A e Luminal B	82 (67,8)	4 (17,4)	2 (33,3)	5 (14,3)	5 (8,8)	
Luminal HER-2	16 (13,2)	5 (21,7)	2 (33,3)	5 (14,3)	11 (19,3)	
Baseline triplo-negative e no baseline triple-negative	23 (19)					

*Values = 0

Tabela 4: Expression associated of marker GATA3 with TNM, immunohistochemistry profile and molecular phenotype of breast cancer.

Variable	GATA3 (% expression associated)					P
	N (%)	0 (Negative)	+	++ (Moderate)	+++ (Strong)	
Staging		36 (30)	14 (11,6)	19 (15,8)	52 (43,3)	0,05
I	32 (26,7)	2 (5,7)	3 (21,4)	8 (42,1)	19 (36,5)	
II	50 (41,7)	17 (48,6)	6 (42,9)	7 (36,8)	20 (38,5)	
III	34 (28,3)	15 (42,9)	5 (35,7)	4 (21,1)	10 (19,2)	
IV	4 (3,3)	1 (2,9)	*	*	3 (5,8)	
Tumor size (cm)						0,019
≤2,0	36 (30)	3 (8,6)	7 (50)	9 (47,4)	17 (32,7)	
2,1 - 5	69 (57,5)	26 (74,3)	7 (50)	7 (36,8)	29 (55,8)	
≥5	15 (12,5)	6 (17,1)	*	3 (15,8)	6 (11,5)	0,548
Distant metastasis						
No	116 (96,7)	34 (97,1)	14 (100)	19 (100)	49 (94,2)	0,261
Yes	4 (3,3)	1 (2,9)	*	*	3 (5,8)	
Nodal status						0,232
0	77 (64,2)	18 (51,4)	9 (64,3)	14 (73,7)	36 (69,2)	
1	24 (20)	8 (22,9)	2 (14,3)	2 (10,5)	12 (23,1)	
2	11 (9,2)	5 (14,3)	3 (21,4)	1 (5,3)	2 (3,8)	
3	8 (6,7)	4 (11,4)	*	2 (10,5)	2 (3,8)	
Grade						0,394
I	16 (13,3)	1 (2,9)	3 (21,4)	5 (26,3)	7 (13,5)	
II	54 (45)	18 (51,4)	7 (50)	8 (42,1)	21 (40,4)	
III	50 (41,7)	16 (45,7)	4 (28,6)	6 (31,6)	24 (46,2)	0,293
RE status						
Positive	89 (74,2)	24 (68,6)	10 (71,4)	17 (89,5)	38 (73,1)	0,242
Negative	31 (25,8)	11 (31,4)	4 (28,6)	2 (10,5)	14 (26,9)	
RP status						0,218
Positive	77 (64,2)	18 (51,4)	9 (64,3)	13 (68,4)	37 (71,2)	
Negative	43 (35,8)	17 (48,6)	5 (35,7)	6 (31,6)	15 (28,8)	0,218
HER-2 status						
Positive	21 (17,5)	9 (25,7)	3 (21,4)	4 (21,1)	5 (9,6)	0,218
Negative	96 (82,5)	26 (74,3)	11 (78,6)	15 (78,9)	47 (90,4)	
Molecular phenotype						0,218
Luminal A e Luminal B	82 (67,8)	22 (61,1)	7 (50)	15 (78,9)	38 (73,1)	
Luminal HER-2	16 (13,2)	8 (22,2)	3 (21,4)	2 (10,5)	3 (5,8)	
Baseline triplo-negative e no baseline triple-negative	23 (19)	6 (16,7)	4 (28,6)	2 (10,5)	11 (21,2)	

*Values = 0

4. Artigo 2

Além do artigo principal referente ao trabalho de mestrado, realizamos outro trabalho intitulado **“EVALUATION OF PAPER AND ELECTRONIC MEDICAL RECORDS IN A TEACHING HOSPITAL FROM SOUTHERN BRAZIL”**, redigido em inglês, segundo as normas da revista Health Education Research.

EVALUATION OF PAPER AND ELECTRONIC MEDICAL RECORDS IN A TEACHING HOSPITAL FROM SOUTHERN BRAZIL

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EVALUATION OF PAPER AND ELECTRONIC MEDICAL RECORDS

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ABSTRACT

Introduction: The recordation of information in medical records by health professionals should be a daily activity. Such documentation contains the data provided by patients and should contain information qualified for use as a teaching tool and for research.

Objective: To ascertain the quality of the information in medical records on the risk factors for breast cancer.

Methods: The presence or absence of the risk factors for breast cancer was verified through a review of the electronic and paper medical records of the patients of a Southern Brazilian teaching hospital.

Results: We evaluated 423 medical records. The greatest loss observed was related to physical exercise, which affected 90% of the records. The most significant loss of information was observed in electronic medical records, compared to paper records, regarding race ($p \leq 0,001$), weight / height (BMI) ($p = 0.01$), alcohol consumption ($p \leq 0,001$), smoking ($p \leq 0,001$), family history of cancer ($p = 0.025$), history of chronic disease ($p \leq 0,001$) and parity ($p \leq 0,001$).

Conclusion: We suggest that the absence of these records may be related to the lack of guidance on how the records must be completed, and that because of this, the information is of poor quality for use as a teaching tool.

Keywords: medical records, education, breast cancer, risk factors, teaching hospital.

INTRODUCTION

The recordation of information in medical records is a daily activity of every health professional; they have a set of data that is provided by patients, and responsible professionals must record that data [1]. It consists of documents of high importance which are aimed at demonstrating the evolution of the condition of the assisted person in order to provide the best therapeutic approach or treatment for that type of patient, in addition to preserving information about the wide variability of preventive care followed by health professionals [1-3]. Thus, an assessment of the quality of the information contained in medical records has great importance, not only as a source of data for research, but also in the context of patient safety [3, 4].

According to the regulatory institutions for health, medical records are valuable not only to the patient, but also to the health team that assists her [5]. Medical records are also valuable for health instruction, teaching, research and public services, in addition to being instruments for legal defence [5]. It is part of the work of health professionals to record information related to their patients' identification and daily evolution, along with the date and time, in paper medical records [1, 5]. In order to be read, the handwriting in a medical record must be legible, and in emergency cases in which is impossible to obtain the patient's medical history, a complete report of all of the diagnostic procedures performed and/or her removal to another unit must be included [1, 5].

Similarly, there are regulations which make up the guidelines on the completeness of anamnesis in electronic medical records, as concern regarding the computerisation of these records has been growing since the 90s [4, 6]. The implementation of electronic media arose through the isolated efforts of students, who tried to develop models for

electronic medical records (EPR) in several institutions, because there was a need for the standardisation of the recordation of information on patients [1, 4, 6].

In order to exemplify the importance of the information contained in these medical records, this study suggests the verification of the quality of such information about the risk factors described in the medical records of patients with breast carcinoma. It is well known that breast cancer is one of the most prevalent types of cancer in the world among women, and in Brazil, it is the neoplasia with the second most common incidence, being responsible for 20% of annual new cases [7]. It is a heterogeneous disease which has different subtypes and unknown etiology, and its development risks are associated with hormonal, hereditary and environmental epidemiologic factors [8-9].

The recordation of information concerning the risk factors in patients' medical records has enormous relevance for the knowledge of the biology and the development of breast carcinomas [10-12]. That is the reason why this model of study was adopted: The variables related to these factors are very important to an understanding of the disease when they are used as an academic research tool.

METHODS

This research is characterised as a transversal study, in which the inclusion criteria for the medical records are: female, older patients diagnosed with breast cancer who were receiving ambulatory care in the referred teaching hospital. A sample size calculation of 400 records was performed, and paper and electronic records were analysed during the period from July 2014 to November 2014, as described in the flowchart in Figure 1. For each medical record in the study, the presence and the absence of information about the risk factors related to breast carcinoma were observed. The variables were classified as:

environmental factors (smoking habits, alcohol intake, practice of physical exercises and weight/height (BMI)), hereditary factors (race, family history of breast cancer and history of chronic diseases) and hormonal factors (menarche, parity, breastfeeding, use of hormones and menopause).

The data were described through absolute and relative frequencies (count and percentage), and the Chi-Square (χ^2) test was used as a statistical test, with a significance level of 5% ($p \leq 0,05$) and a confidence interval of 95%. The processing and analysis of the data were executed using the software Statistical Package for the Social Sciences (SPSS 21® - Chicago, USA).

The study was conducted according to Resolution 466/12 of the National Council of Health, respecting the ethical and legal requirements for the anonymity and secrecy of the patients' information, with the approval of the Ethics Committee of the institution where this study was performed and the Declaration of Helsinki, 2013. The authors declare that there are no conflicts of interest.

RESULTS

We evaluated a total of 423 records, and of these, 267 were paper and 156 were electronic. In the information related to the risk factors in all of the medical records analysed, we could verify that most of the data losses, 90%, were among those referring to the practice of physical exercises, and the losses were more common in the electronic records (Table 1). This is followed by losses of information about breastfeeding, 71.9% ($n=304$), the use of hormones, 61.9% ($n=262$) and race, 56% ($n=237$), which were also not found.

In table 1, the total score distribution observed in both types of medical records was registered. In the paper medical records, the largest losses of information concerned the following risk factors: the practice of physical exercise ($p \leq 0.001$), breastfeeding ($p \leq 0.001$), the use of hormones ($p \leq 0.001$) and menopause ($p = 0.032$).

With respect to the information that was contained in the same records, the *presence* of the following risk factors was significant: smoking habits ($p \leq 0.001$), weight/height (BMI) ($p \leq 0.001$), a family history of breast cancer ($p = 0.002$) and a history of chronic disease ($p \leq 0.001$).

In the analysis of the electronic medical records, it was observed that the most significant *losses* involved information concerning race ($p \leq 0.001$), weight/height (BMI) ($p = 0.01$), alcohol intake ($p \leq 0.001$), and similar to the paper records, the practice of physical exercise ($p \leq 0.001$) and breastfeeding ($p \leq 0.001$). However, the information found in the electronic records was more significant when it was related to smoking habits ($p \leq 0.001$), a family history of breast cancer ($p = 0.025$), a history of chronic disease ($p \leq 0.001$) and parity ($p \leq 0.001$) (Table 1).

DISCUSSION

The main focus of this study was an investigation of the presence and absence of information about the risk factors for breast cancer in the medical records of a teaching hospital. The quality of this information for use as an auxiliary teaching tool was also evaluated. Thus, the study found that the lack or poor quality of this information has been and continues to be an obstacle in the development of investigations about the disease in the academic environment, which reinforces other studies that have shown the great value of these registers for health research and teaching [2, 13, 14].

With respect to the investigated risk factors, the most frequent and significant loss of information in both types of medical records (electronic and paper-based) occurred regarding the environmental factor of the practice of physical exercise. In a recent study of European women, there was evidence that physical inactivity is linked to the premature death of patients [15]. This study showed a strong relationship between the predisposition of developing cardiovascular disease and cancer, independent of BMI, in the individuals presented [15]. According to some studies that have established the relevance of the standardisation of information for data queries in scientific circles, the failure to register this risk factor may be linked to a lack of guidance on the completion of hospital records [13, 16-18]. Therefore, it is emphasised that this information has great value for scientific research related to the disease.

By comparing the information contained in paper and electronic medical records, it was found that the most significant losses occurred in the surveyed electronic medical records. Studies have shown that the implementation and compliance with the EP (electronic medical records) by hospitals and health professionals is still deficient and has experienced difficulties [19-21, 23]. The accessioning of records has become a major challenge linked to teaching and the daily practice of qualified information registry, which includes clinical research [19-21, 23].

In the paper records, it was found that there were more complete records regarding the risk factors studied, excluding physical exercise, breastfeeding, the use of hormones and menopause. In the electronic medical records, among the variables studied, the information that is meaningful but absent includes: for environmental factors, physical exercise, intake of alcohol and BMI; for hereditary factors, race and family history of cancer; and for hormonal factors, parity and breastfeeding. Some findings have shown that there may be resistance by some health professionals related to the correct completion

of the information in an electronic format. This information corroborates the existing literature, wherein some studies have mentioned difficulties related to the completion of the records, which is reflected in the poor quality of the scientific information found in them [23, 24]. It is suggested that in order to obtain the full use of these records as an educational tool for academic training and to use the information in our database, the use of mandatory fields that must be filled in during the recordation of patients' medical history in electronic media would be an important option to avoid the loss of information.

CONCLUSION

In the performance of this work, it is understandable that instruction in a teaching hospital is directly related to the medical history records that are held by health professionals. Moreover, in teaching hospitals, in which students complete the medical records under supervision, correcting them becomes the responsibility of the health care provider.

The implementation of electronic medical records in those teaching hospitals, along with innovations in the educational system in health-related areas, may act as alternatives that can facilitate the standardisation and the quality of the sets of data provided by patients and may also make it easier to register such data sets in their medical records. However, it is important to note that the implementation of this new database is struggling, because the consent and adherence of the patients still requires substantial effort on the part of both health professionals and academia. The difficulty in implementing the electronic system ultimately harms the excellence of the information recorded about patients in their medical records, and therefore, its implementation should be part of the courses of learning that are foundational in health.

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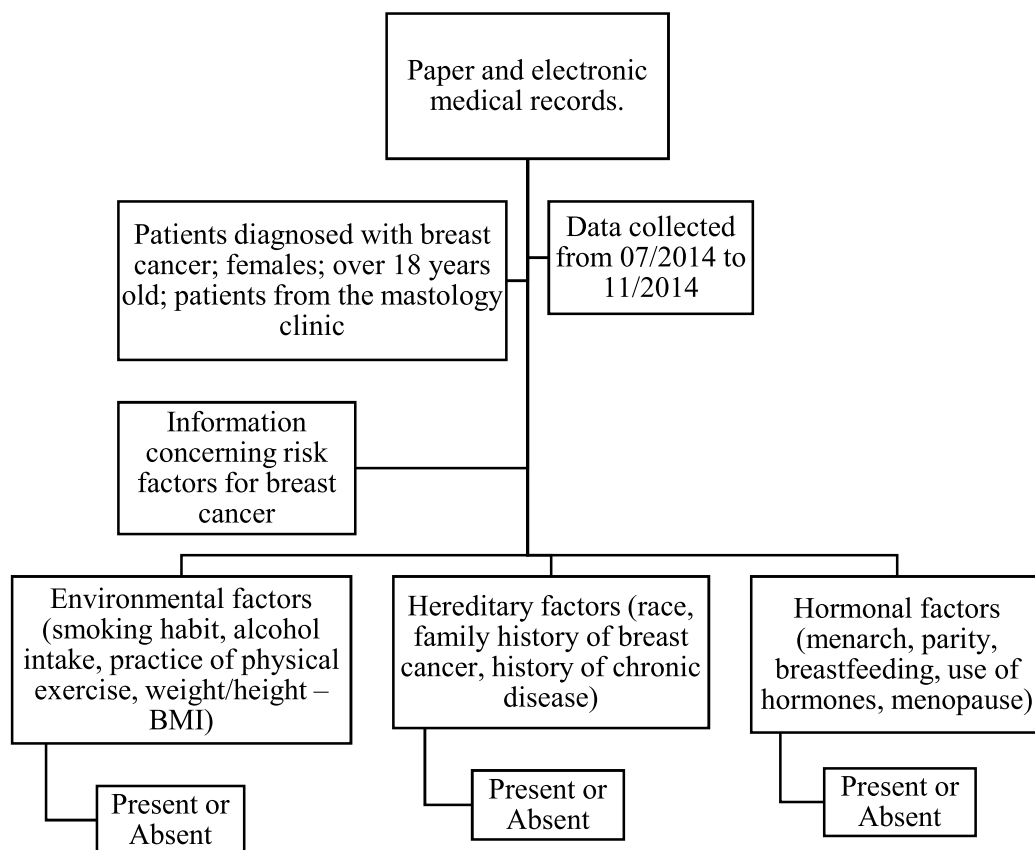


Figure 2: Schematic flowchart about the study's methodology.

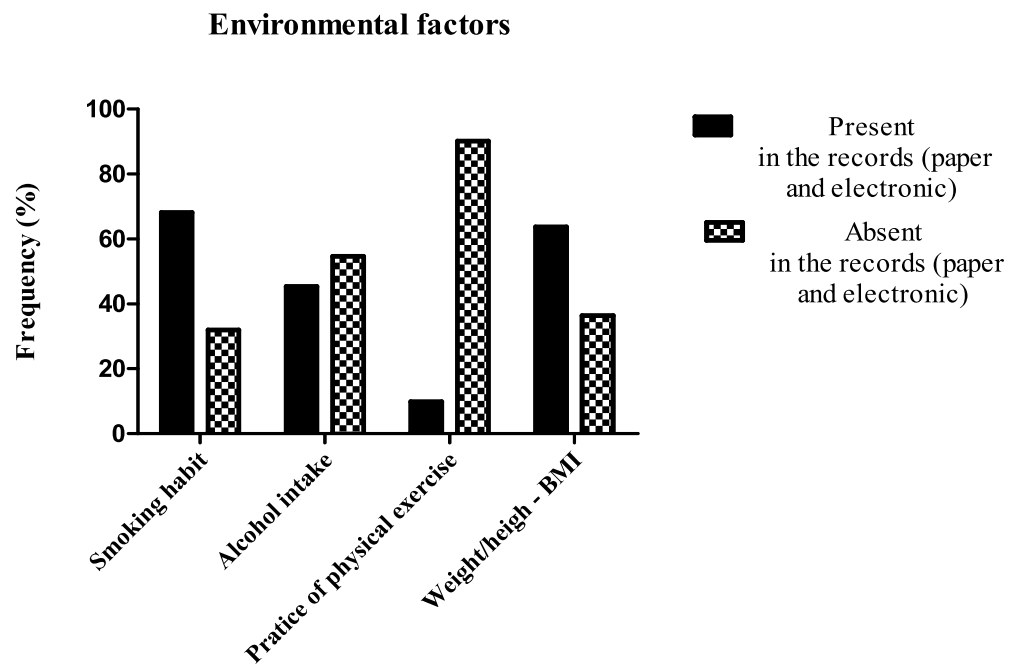


Figure 3 Environmental risk factors had greater loss of information in the evaluation of the records.

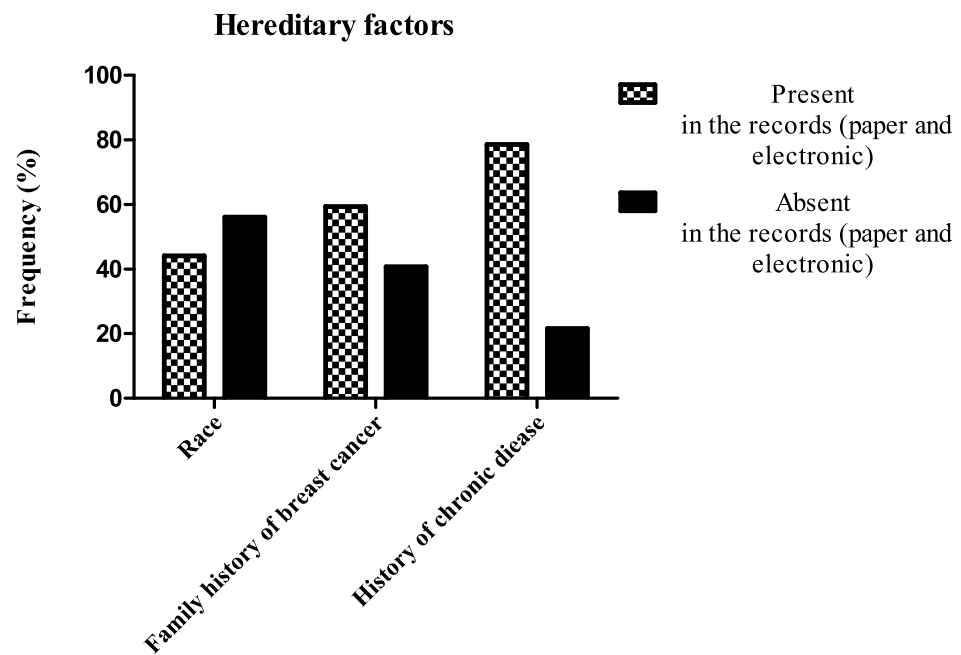


Figure 4 Hereditary risk factors that had greater loss of information in the evaluated records.

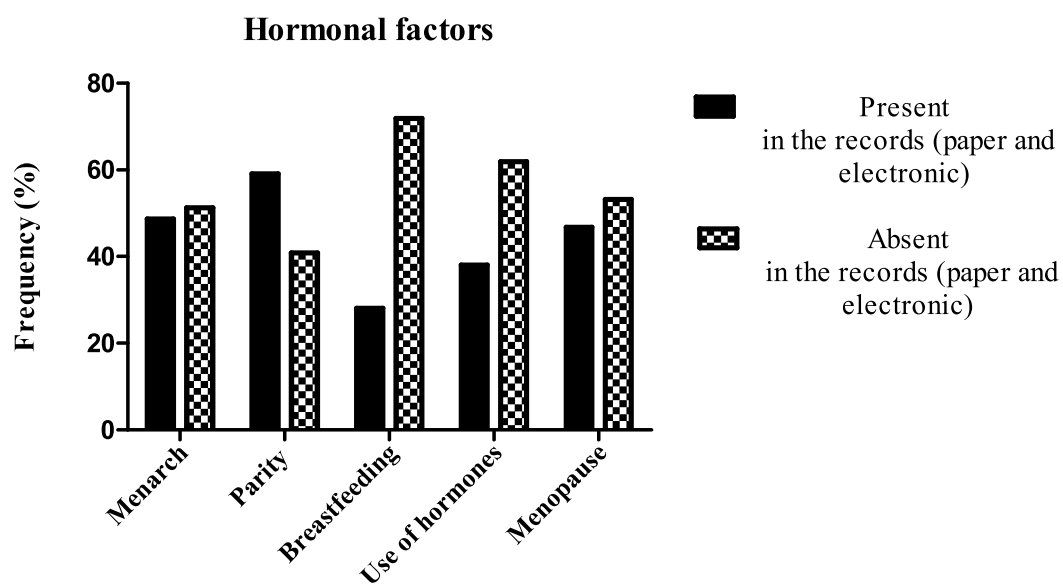


Figure 4. Hormonal risk factors that had greater loss of information in the evaluated records.

Table 1 Statistical correlation between the information about breast cancer risk factors found in paper and electronic records.

Significant p value $\leq 0,05$.

Risk Factors	Information	Paper records			Electronic records		
		n	%	<i>P value</i>	n	%	<i>P value</i>
<i>Environmental</i>							
Smoking habit	<i>Present</i>	185	69,3	≤ 0,001	103	66	≤ 0,001
	<i>Absent</i>	82	30,7		53	34	
Alcohol intake	<i>Present</i>	140	52,4	0,426	52	33,3	≤ 0,001
	<i>Absent</i>	127	47,6		104	66,7	
Practice of physical exercise	<i>Present</i>	23	8,6	≤ 0,001	19	12,2	≤ 0,001
	<i>Absent</i>	244	91,4		137	87,8	
Weight/height - BMI	<i>Present</i>	207	77,5	≤ 0,001	62	39,7	0,01
	<i>Absent</i>	60	22,5		94	60,3	
<i>Hereditary</i>							
Race	<i>Present</i>	173	64,8	≤ 0,001	13	8,3	≤ 0,001
	<i>Absent</i>	94	35,2		143	91,7	
Family history of breast cancer	<i>Present</i>	159	59,6	0,002	92	59	0,025
	<i>Absent</i>	108	40,4		64	41	
History of chronic disease	<i>Present</i>	211	79	≤ 0,001	121	77,6	≤ 0,001
	<i>Absent</i>	56	21		35	22,4	
<i>Hormonal</i>							
Menarche	<i>Present</i>	132	49,4	0,854	74	47,4	0,522
	<i>Absent</i>	135	52,6		82	52,6	
Parity	<i>Present</i>	147	55,1	0,098	103	66	≤ 0,001
	<i>Absent</i>	120	44,9		53	34	
Breastfeeding	<i>Present</i>	82	30,7	≤ 0,001	37	23,7	≤ 0,001
	<i>Absent</i>	185	69,3		119	76,3	
Use of hormones	<i>Present</i>	91	34,1	0,001	70	44,9	0,2
	<i>Absent</i>	176	65,9		86	55,1	
Menopause	<i>Present</i>	116	43,4	0,032	82	52,6	0,522
	<i>Absent</i>	151	56,6		74	47,4	
<i>Total</i>		267			156		

5. Artigo 3

Participação com co-autoria no artigo intitulado **“A imunoexpressão e a ocorrência de HPV em pacientes transplantados renais a partir de exame citológico urinário”**, aceito para publicação no Jornal Brasileiro de Patologia e Medicina Laboratorial.

Immunosuppression and the occurrence of HPV in kidney transplant patients verified by urinary cytology

Imunossupressão e ocorrência de HPV em pacientes transplantados renais a partir de exame citológico urinário

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ABSTRACT

Introduction: Human papillomavirus (HPV) is the main cause of cervical cancer, and immunosuppression is recognized as a risk factor for HPV infection and its persistence. After renal transplantation, immunosuppressive agents are used to prevent rejection, but predispose recipients to chronic infections and malignancies. **Objective:** This study aimed to verify, based on urinary cytology (UC), the prevalence of HPV in immunosuppressed kidney transplant patients. **Material and method:** In this cross-sectional study, the population was composed of kidney transplant patients that had undergone routine UC from August 2012 to August 2014. **Results:** There were 2,305 urine cytological tests. Thirteen patients with presence of koilocytes in such examination were observed. Therefore, the relative frequency of patients with HPV detected in urine was 0.56%. In the interval until the first post-transplant year, 10 (76.92%) patients presented koilocytes ($p < 0.0001$) in the UC. The dosages of immunosuppressive agents until the first post-transplant consultation, which showed correlation with the period between transplantation and the first UC test with the presence of koilocytes ($p < 0.0001$), were: prednisone 10.5-20 mg/day, mycophenolate sodium 901-1,440 mg/day, and tacrolimus 4.5-12 mg/day. **Conclusion:** This study showed immunosuppression as an important risk factor for infection by HPV or its reactivation. Screening UC tests after transplantation may evidence HPV infection.

Key words: immunosuppression; risk factors; kidney transplantation; papillomavirus infections.

INTRODUCTION

Human papillomavirus (HPV) is the main cause of cervical cancer among women, and immunosuppression is recognized as one of the risk factors for HPV infection and its persistence^{1,2}. According to Meersow *et al.* (2011), HPV prevalence is higher in immunosuppressed women in comparison to their immunocompetent homologues. Immunosuppression probably causes greater susceptibility to HPV infection and/or higher risk of HPV infection persistence³.

Kidney transplant recipients have high risk of developing premalignant neoplasms associated with the virus, such as HPV-related anogenital lesions. Most kidney transplant women are known to be infected by HPV, with a 14-fold higher risk of developing cervical cancer, 50-fold higher risk for vulvar cancer, and up to 100-fold higher risk for anal cancer⁴.

Recognizing HPV infection, by the cell alteration (koilocytosis) observed when this virus is present, became important due to its identification as carcinogenic in the development of squamous cell carcinoma of the genital tract⁵. During the viral course of HPV, viral particles invade the cell nucleus and cause characteristic degenerative alterations⁶. Koilocytosis is considered a pathognomonic sign of HPV infection. The typical koilocytosis presents nuclear enlargement, with reactive changes, and characteristic sharply demarcated halo separated from cytoplasm by a clearly condensed rim⁴.

As reported by Reis *et al.* (2010), HPV infection is frequently common among young adults of both sexes, with estimated prevalence of 20%-40%. HPV dissemination is generally universal among sexually active individuals, with men playing an important role in spreading this virus among women⁷.

Infection by specific HPV types is the main risk factor for cervical cancer. Other risk factors are early age of first sexual intercourse,

6. Considerações finais

Com base nos resultados encontrados, pode-se concluir que houve uma associação significativa entre a expressão dos marcadores UGT8 e GATA3 ao tamanho tumoral, expressão de RP e estadiamento dos carcinomas primários de mama estudados.

No entanto, sugere-se que novas pesquisas com esses potenciais marcadores devem ser realizadas, pois possuem importância no entendimento do processo de desenvolvimento metastático e prognóstico da doença.

Também se observou que a maior limitação desta pesquisa esteve relacionada ao tamanho amostral, uma vez que houveram perdas amostrais, como blocos de anatomopatológico não encontrados.

Salienta-se que, a partir do mestrado, surgiram novos trabalhos, como o artigo intitulado “Avaliação dos Prontuários de Papel e Eletrônico de Hospital Escola no Sul Do Brasil”, submetido para publicação, bem como, parceria na coautoria de outro estudo relacionado à infecção por HPV em transplantados renais, intitulado “A imunoexpressão e a ocorrência de HPV em pacientes transplantados renais a partir de exame citológico urinário”, aceito para publicação no Jornal Brasileiro de Patologia e Medicina Laboratorial.

ANEXOS

Anexo I - Classificação dos tumores da mama segundo a Organização Mundial da saúde, 2012.

Tabela 2: Classificação dos tumores da mama segundo a Organização Mundial da saúde, 2012: Tumores epiteliais, epiteliais raros, lesões proliferativas intraductais e lesões precursoras.

Tumores epiteliais	Tumores epiteliais raros	Lesões proliferativas intraductais e lesões precursoras
Carcinoma microinvasivo Carcinoma mamário invasivo Carcinoma ductal invasivo, SOE Carcinoma tipo misto Carcinoma pleomórfico Carcinoma com células gigantes tipo osteoclasto Carcinoma com elementos coriocarcinomatosos Carcinoma com elementos melanóticos Carcinoma lobular invasivo Carcinoma lobular clássico Carcinoma lobular sólido Carcinoma lobular alveolar Carcinoma lobular pleomórfico Carcinoma túbulo-lobular Carcinoma lobular misto Carcinoma tubular Carcinoma cribriforme invasivo Carcinoma com elementos medulares Carcinoma medular Carcinoma medular atípico Carcinoma invasivo SOE com elementos medulares Carcinoma mucinoso Carcinoma com diferenciação em células em anel de sinete Carcinoma micropapilar invasivo Carcinoma com diferenciação apócrina Carcinoma metaplásico sem tipo especial Carcinoma adenoescamoso de baixo grau Carcinoma metaplásico fibromatoso-símil Carcinoma de células escamosas Carcinoma de células fusiformes Carcinoma metaplásico com diferenciação mesenquimal Diferenciação condroide Diferenciação óssea Diferenciação em outros tipos mesenquimais Carcinoma metaplásico misto	Carcinoma com elementos neuroendócrinos Tumor neuroendócrino bem diferenciado Carcinoma neuroendócrino pouco diferenciado (carcinoma de pequenas células) Carcinoma com diferenciação neuroendócrina Carcinoma secretor Carcinoma papilar invasivo Carcinoma de células acinares Carcinoma mucoepidermoide Carcinoma oncocítico Carcinoma rico em lípidos Carcinoma de células claras rico em glicogénio Carcinoma sebáceo Tumores tipo glândula salivariáneas cutâneas Cilindroma Hidroadenoma de células claras	Lesões precursoras Carcinoma ductal in situ Neoplasia lobular Carcinoma lobular in situ Carcinoma lobular in situ clássico Carcinoma lobular in situ pleomórfico Hiperplasia lobular atípica Lesões proliferativas intraductais Hiperplasia ductal usual Lesões de células colunares Atipia epitelial plana Hiperplasia Hiperplasia ductal atípica Carcinoma ductal in situ

SOE: sem outra especificação;

*Na 3ª edição, esses tumores eram listados entre os carcinomas invasivos, sem chamar a atenção para sua raridade.

(Adaptado de Gobbí, 2012 - Classificação dos tumores da mama: atualização baseada na nova classificação da Organização Mundial da Saúde de 2012 e OMS, 2012 - WHO Classification of Tumours, Volume 4).

Tabela 3: Classificação dos tumores da mama segundo a Organização Mundial da saúde, 2012: Tumores epiteliais/mioepiteliais, lesões e neoplasias papilares, proliferações epiteliais benignas e tumores mesenquimais.

Tumores epiteliais/mioepiteliais	Lesões e neoplasias papilares	Proliferações epiteliais benignas	Tumores mesenquimais
Adenoma pleomórfico	Papiloma intraductal	Adenose esclerosante	Fasciite nodular
Adenomioepitelioma	Papiloma intraductal com hiperplasia atípica	Adenose apócrina	Miofibroblastoma
Adenomioepitelioma com carcinoma	Papiloma intraductal com carcinoma ductal in situ	Adenose microglandular	Fibromatose tipo desmoide
Carcinoma adenoide cístico	Papiloma intraductal com carcinoma lobular in situ	Cicatriz radial/lesão esclerosante complexa	Tumor miofibroblástico inflamatório
	Carcinoma papilífero intraductal	Adenomas	Lesões vasculares benignas
	Carcinoma papilífero encapsulado	Adenoma tubular	Hemangioma
	Carcinoma papilífero encapsulado com invasão	Adenoma lactante	Angiomatose
	Carcinoma papilífero sólido	Adenoma apócrino	Lesões vasculares atípicas
	In situ	Adenoma apócrino	Hiperplasia pseudoangiomatosa do estroma
	Invasivo	Adenoma ductal	Tumor de células granulares
			Tumores benignos da bainha de nervo periférico
			Neurofibroma
			Schwanoma
			Lipoma
			Angiolipoma
			Lipossarcoma
			Angiossarcoma
			Rabdomiossarcoma
			Osteossarcoma
			Leiomioma
			Leiomiossarcoma

* Na 3ª edição, o carcinoma papilífero invasivo foi incluído entre os carcinomas invasivos e, na 4ª edição, entre os tipos raros de tumores da mama. OMS: Organização Mundial da Saúde.

(Adaptado de Gobbi, 2012 - Classificação dos tumores da mama: atualização baseada na nova classificação da Organização Mundial da Saúde de 2012 e OMS, 2012 - WHO Classification of Tumours, Volume 4).

Anexo II – Parecer Consusubstanciado do Comitê de Ética em Pesquisa.

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA	
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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estudo dos marcadores tumorais no câncer de mama.

Pesquisador: Claudia Giuliano Elia

Área Temática:

Versão: 2

CAAE: 22396713.1.0000.5335

Instituição Proponente: Irmandade da Santa Casa de Misericórdia de Porto Alegre - ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Numero do Parecer: 525.170

Data da Relatoria: 22/01/2014

Apresentação do Projeto:

A proposta da presente pesquisa apresenta dois desenhos, subdivididos de acordo com a metodologia a ser adotada, sendo um estudo transversal e um estudo de coorte prospectiva. O câncer de mama trata-se de uma doença heterogênea que possui uma etiologia complexa. Nos últimos anos o câncer de mama foi considerado um problema de saúde pública, sendo o segundo tipo de neoplasia mais frequente no mundo e o de maior frequência entre as

mulheres. As alterações no estilo de vida, bem como a adoção de hábitos não saudáveis da população, podem ajudar a responder o aumento de casos de câncer na população mundial. O advento da tecnologia de microarrays de DNA complementar, com a análise paralela de milhares de genes, tem permitido correlacionar perfis de expressão gênica dos cânceres de mama com a evolução clínica das pacientes e com as respostas às terapias utilizadas. Painéis imunohistoquímicos têm sido propostos para a identificação desses subtipos, buscando reproduzir com certa aproximação os perfis de expressão gênica. Essas descobertas têm proporcionado importantes informações prognósticas e preditivas e uma melhor percepção sobre os complexos mecanismos biológicos da tumorigênese. Tamanho da amostra de 1320 pacientes.

Objetivo da Pesquisa:

Objetivo: Investigar marcadores preditivos do câncer de mama.

Endereço: R. Profª Anna Dias, 285 Hosp. Dom Vicente Scherer			
Bairro: 6º andar - Centro		CEP: 90.020-090	
UF: RS	Município: PORTO ALEGRE		
Telefone: (51)3214-8511	Fax: (51)3214-8571	E-mail: cep@santacasa.lche.br	

Anexo III – Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Título do Projeto: “Estudo dos tumores no câncer de mama”

Você está sendo convidada a participar como voluntária deste estudo, pois apresentou câncer de mama. O objetivo desta pesquisa é estudar marcadores tumorais, substâncias presentes no organismo, que podem nos trazer informações relevantes sobre o comportamento do câncer. Essas substâncias estão presentes em diversos locais do seu corpo, como no sangue e no tumor, os quais denominados de Materiais Biológicos.

Para participar desta pesquisa será necessário que você nos autorize consultar seu prontuário no hospital, a ceder uma amostra do seu material biológico (tumor ou líquido) que está sob guarda do laboratório de patologia, e caso você se sinta em condições e queira colaborar além do que já foi solicitado acima, pedimos uma amostra de sangue (3 ml) para podermos utilizar em pesquisas, relacionadas exclusivamente a este estudo proposto.

(☐) SIM, quero doar também o sangue. (Risco: desconforto e as vezes hematomas com a picada)

(☐) NÃO quero doar o sangue, mas participarei deste estudo, a partir do material do Laboratório e consulta no prontuário.

O seu material será armazenado no Laboratório de Pesquisa em Patologia da UFCSPA, sendo os mesmos armazenados pela pesquisadora principal durante 5 (cinco) anos. Após este período, os blocos serão devolvidos ao laboratório de referência e o sangue será desprezado. (conforme preconiza a Resolução 466/12).

Afirmamos que se você se sentir por qualquer motivo, desconfortável em participar deste estudo, você poderá desistir a qualquer momento, pedindo a retirada de consentimento, sem que isso represente qualquer forma de prejuízo em seu tratamento. A sua participação é inteiramente voluntária.

Informamos que você não será identificável em nenhum momento da pesquisa, garantindo assim o seu anonimato, não estabelecendo nenhuma relação de diagnóstico, pois este projeto possui exclusivamente caráter científico.

Informamos que este estudo não envolverá custos para você e que, da mesma forma, não haverá remuneração por participar desta pesquisa. O seu benefício dar-se-á por

Termo de Consentimento Livre e Esclarecido (TCLE) - Versão 1.0 de 04 de Outubro de 2013

2

meio dos resultados científicos desta pesquisa após a conclusão do projeto. Você não se beneficiará imediatamente deste estudo, entretanto o conhecimento adquirido poderá contribuir para o melhor entendimento da sua doença.

Eu _____, recebi as informações sobre os objetivos e a importância desta pesquisa de forma clara e concordo em participar do estudo. Declaro que também fui informado:

Da garantia de receber resposta a qualquer pergunta ou esclarecimento acerca dos assuntos relacionados a esta pesquisa;

De que minha participação é voluntária e terei a liberdade de retirar o meu consentimento, a qualquer momento, sem que isto traga prejuízo para a minha vida pessoal e nem para o atendimento prestado a mim.

Da garantia que não serei identificado quando da divulgação dos resultados e que as informações serão utilizadas somente para fins científicos de pesquisa.

Sobre o projeto de pesquisa e a forma como será conduzido e que em caso de dúvida ou novas perguntas poderei entrar em contato com a pesquisadora principal Claudia G. Bica, no telefone 3303-8760, onde terei o contato através da via do TCLE que ficará comigo.

Também que, se houverem dúvidas quanto a questões éticas, poderei entrar em contato com o Comitê de Ética em Pesquisa da Santa Casa de Misericórdia de Porto Alegre pelo telefone 3214-8571, endereço Rua Prof. Annes Dias, Centro, Porto Alegre, RS; Comitê de Ética em Pesquisa da UFCSPA, Rua Sarmento Leite, 245, Centro, Porto Alegre, RS pelo telefone 3303-8804 e Daniel Demétrio Faustino da Silva, Coordenador-geral do Comitê de Ética em Pesquisa do GHC pelo telefone 3357-2407, endereço Av. Francisco Trein 596, 3º andar, Bloco H, sala 11, das 09h às 12h e das 14h-30min às 17h.

Caso tiver novas perguntas sobre este estudo, posso chamar a Pesquisadora responsável pelo Estudo, Claudia G. Bica telefone (51) 3303-8760.

Nome do participante por extenso (letra de forma)

Data da assinatura do termo

Assinatura do participante

"Declaro que esclareci os objetivos do projeto de forma clara, da justificativa e dos procedimentos a que serão submetidos, bem como dos riscos, desconfortos e benefícios do presente trabalho. Também discuto e esclareço dúvidas sobre a pesquisa. Atesto que assino e entrego uma cópia idêntica do termo de consentimento ao participante".

Nome do pesquisador que aplicou o TCLE (letra de forma)

Data da aplicação do termo

Assinatura do pesquisador que aplicou o TCLE e carimbo.

Estudo dos Marcadores Tumoriais do Câncer de Mama - Questionário

Questionário estruturado para obtenção de dados a respeito de fatores de risco, cirurgia e tratamento no câncer de mama, aplicado às pacientes com a doença, atendidas no Ambulatório de Mastologia - SUS, no Hospital Santa Rita do Complexo Hospitalar Santa Casa de Misericórdia de Porto Alegre.

*Obrigatório

1- Prontuário *

2- Nome da Paciente *

3- Data de Nascimento *

4- Idade *

Em meses

5- Idade no momento do diagnóstico *

Em meses

6- Município em que reside

1- Pou 2- Grande Pou 3- Interior

☐ 1

☐ 2

☐ 3

7- Menarca

1- antes dos 12 anos, 2- 12 - 14 anos, 3- 15 anos, ou mais 4- Não sabe 5- Recusou-se a responder

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

8- Raça

1- Branca 2- Negra 3- Parda 4- Amarela 5- Indígena 6- Não informado

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

☐ 6

9- Paridade:

1- Sim 2- Não 3- Recusou-se a responder

☐ 1

☐ 2

☐ 3

10- Número de Filhos

11- Amamentou

1- Sim 2- Não 3- Recusou-se a responder

☐ 1

☐ 2

☐ 3

12- Anticoncepcional

1- Sim 2- Não 3- Recusou-se a responder

☐ 1

☐ 2

☐ 3

13- Tempo de uso de Anticoncepcional

1- menos de 5 anos 2- 5-10 anos 3- 10 anos, ou mais 4- Não sabe 5- Recusou-se a responder

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

14- Menopausa

1- Sim 2- Não 3- Não sabe 4- Recusou-se a responder

☐ 1

☐ 2

☐ 3

☐ 4

15- Idade que entrou na menopausa

1- Antes dos 40 anos 2- 40-55 anos 3- Após 55 anos 4- Não sabe 5- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5

16- Reposição Hormonal

1- Sim 2- Não 3- Não sabe 4- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4

17- Tempo de reposição

1- menos de 1 ano 2- 1-2 anos 3- 3-4 anos 4- 5 anos ou mais 5- Não sabe 6- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5

18- Peso atual

Números inteiros

19- Peso antes

Números inteiros

20- Altura

Em centímetros

21- Exercícios

1- Sim 2- Não 3- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3

22- Tabagismo

1- Sim 2- Não 3- Recusou-se a responder

23- Tempo de tabagismo

1- menos de 1 ano 2- 1-2 anos 3- 3-4 anos 4- 5 anos, ou mais 5- Não sabe 6- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5

24- Álcool

1- Sim 2- Não 3- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3

25- Histórico de doença crônica

1- Sim 2- Não 3- Não sabe

- ☐ Hipertensão
☐ Diabetes
☐ Colesterol alto
☐ Triglicéridos alto
☐ Hiper ou hipotireoidismo
☐ Outra
☐ Outro:

26- História de CA MAMA

1- Sim 2- Não 3- Não sabe 4- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4

27- Parentesco CA MAMA

1- 1º grau 2- 2º grau 3- 3º grau

- ☐ 1
☐ 2
☐ 3

28- História Familiar de OUTRO TIPO DE CÂNCER

1- Sim 2- Não 3- Não sabe 4- Recusou-se a responder

- ☐ 1

28- Parentesco História Familiar de OUTRO TIPO DE CÂNCER

1- 1º grau 2- 2º grau 3- 3º grau

- ☐ 1
☐ 2
☐ 3

29- Sinal de alerta

1- Carcoço na axila 2- Sangue mamilo 3- Dor na mama 4- Alt. Tam. Mama 5- Irreg pele 6- Ret/Dep Mamilo 7- Alt. cor da mama 8- Nódulo 9- Ardor/queimação 10- Alteração na mamo/eco

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10

30- Cirurgia

1- Sim 2- Não 3- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3

31- Data diagnóstico

Mês e ano

32- Data da cirurgia

Mês e ano

32a- Tempo de cirurgia

em meses

33- Cirurgia realizada

1- Ambas mamas total 2- Ambas mamas parcial 3- Uma mama total 4- Uma mama parcial 5- Recusou-se a responder

34- Metástase

1- Não 2- Não sabe 3- Recusou-se a responder 4- Recidiva local 5- Na outra mama 6- Pulmão 7- Osso 8- Cérebro 9- Fígado 10- Linfonodos 11- Dérmica próxima a mama 12- Peritônio 13- Outros

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ 11
☐ 12
☐ 13

35- Tratamento CA MAMA

1- Sim 2- Não 3- Não sabe 4- Recusou - se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4

36- Quimioterapia

1- Sim 2- Não 3- Recusou - se a responder

- ☐ 1
☐ 2
☐ 3

37- Tipo de QUIMIO

1- Pré-oper 2- Pós-oper 3- Pré e pós 4- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4

38- Ciclos da Quimio

39- Radioterapia

1- Sim 2- Não 3- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3

40- Sessões de Radio

1- 10 - 20 sessões 2- 20-30 sessões 3- mais de 30

- ☐ 1
☐ 2
☐ 3

41- Hormonioterapia

1- Sim 2- Não 3- Não sabe 4- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4

42- Tratamento METÁSTASE

1- Não 2-Químio 3- Radio 4- Químio e Radio 5- Cirurgia 6- Cirurgia + químio 7- Cirurgia + Radio 8- Cirurgia + Químio + Radio 9- Não sabe 10- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10

43- Tratamento alternativo

1- Sim 2- Não 3- Recusou-se a responder

- ☐ 1
☐ 2
☐ 3

43a- Se sim, qual?



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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

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DESCRIPTION

The Breast: Aims and Scope

The Breast is an international, multidisciplinary journal for researchers and clinicians, which focuses on translational and clinical research for the advancement of breast cancer prevention, diagnosis and treatment of all stages. The Editors welcome the submission of original research articles, systematic reviews, and viewpoint/commentary and debate articles, and correspondence on all areas of pre-malignant and malignant breast disease, including:

- Epidemiology and prevention
- Translational research, encompassing the use of new technologies, molecular biology, genetics and pathology
- Screening, early diagnosis, follow-up and response assessment: use of imaging, nuclear medicine and other technologies
- Medical oncology
- Radiation oncology
- Breast surgery
- Psycho-oncology
- Quality of life
- Survivorship
- Supportive care
- Palliative and end-of-life care
- Advocacy
- Breast Nursing
- Breast Units management and organization of breast care, including health economics

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GUIDE FOR AUTHORS

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To find out more, please visit the Preparation section below.

INTRODUCTION

The Breast is an international, multidisciplinary journal for clinicians, which focuses on translational and clinical research for the advancement of breast cancer prevention and therapy. The Editors welcome the submissions of original research articles, systematic reviews, viewpoint and debate articles and correspondence on all areas of pre-malignant and malignant breast disease.

General

The Breast will consider manuscripts prepared according to the guidelines adopted by the International Committee of Medical Journal Editors ("Uniform requirements for manuscripts submitted to biomedical journals", available as a PDF from <http://www.icmje.org>).

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BEFORE YOU BEGIN

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No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. **All animal studies need to ensure they comply with the ARRIVE guidelines.** More information can be found at <http://www.nc3rs.org.uk/page.asp?id=1357>.

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Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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1. Cochran R, Gee A, Ellis H. Microscopic topography of the male breast. *The Breast* 1992; 1: 25-27.
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SCOPE

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2. Fynn A. Cigarette advertising and health education: use and abuse of media. In: Leather DS, Hastings GB, Davies JK (eds). *Health Education and the Media*. Oxford: Pergamon Press, 1981. 129.
3. Begler R. *Advertising and Cigarette Smoking: a Psychological Study*. Bern: Hans Huber, 1981.
4. Paul CL, Redman S, Sanson-Fisher RW. A cost-effective approach to the development of printed materials: a randomized controlled trial of three strategies. *Health Educ Res* May 20, 2004; 10:1093/*her/cyg090*.
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