UNIVERSIDADE FEDERAL DE VIÇOSA

PERFIL DOS ÁCIDOS GRAXOS DA DIETA E DO TECIDO ADIPOSO DA MAMA, POLIMORFISMOS E RISCO DE DOENÇA BENIGNA E CÂNCER DE MAMA

Lisiane Lopes da Conceição
Doctor Scientiae.

VIÇOSA
MINAS GERAIS – BRASIL
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"Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes".

(Marthin Luther King)

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BIOGRAFIA

LISIANE LOPES DA CONCEIÇÃO, filha de Gilcemir Angelo da Conceição e Maria do Carmo Duarte Lopes da Conceição, nasceu no dia três de novembro de 1986 em Viçosa, estado de Minas Gerais.

Em março de 2005, ingressou no curso de Nutrição da Universidade Federal de Viçosa (UFV), graduando-se como Nutricionista em janeiro de 2010.

Em agosto de 2010, iniciou, na mesma instituição, no Programa de Pós-Graduação em Microbiologia Agrícola, nível mestrado, na área de Microbiologia Industrial, concluído em 2012.

Em março de 2013, ingressou no curso de Doutorado do Programa de Pós-Graduação em Ciência da Nutrição da Universidade Federal de Viçosa, concentrando seus estudos na área de valor nutricional, funcional e controle de qualidade de alimentos e dietas.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

AGST	Àcidos Graxos Saturados Totais
DNA	Ácido Desoxirribonucleico
BBD	Benign Breast Disease
BC	Breast Cancer
BMI	Body Mass Index
C	Control Group
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CI 95%	Confidence Intervals of 95%
CM	Câncer de mama
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DBM	Doença Benigna da Mama
ER-α	Receptor de Estrogênio alfa
ER-β	Receptor de Estrogênio beta
FA	Fatty Acid
FAPEMIG	Fundação de Amparo à Pesquisa de Minas Gerais
FHEMIG	Hospital Foundation of Minas Gerais
GBBD	Group Benign Breast Disease
GC	Group Control
MV	Mimetismo Vasculogênico
MUFA	Total Monounsaturated Fatty Acids
MTHFR	Metilenotetrahidrofolato Redutase
MTR	Metionina Sintetase
NF-kB	Fator Nuclear kappa B
OR	*odds ratios*
PAQ	International Physical Activity Questionnaire
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction - Restriction Fragment Length Polymorphism Analysis
PUFA	Polyunsaturated Fatty Acids

PPARγ	Receptor Ativado por Proliferadores de Peroxissomos gama
SCD1	Enzyme Stearoyl-CoA Desaturase-1
SFA	Saturated Fatty Acids
SFFQ	Semiquantitative Food Frequency Questionnaire
SHR	*Spontaneously Hypertensive Rat*
SNP	*Single Nucleotide Polymorphisms*
WHR	Waist to Hip Ratio
WHtR	Waist to Height Ratio

RESUMO

CONCEIÇÃO, Lisiane Lopes da, D.Sc., Universidade Federal de Viçosa, novembro de 2016. **Perfil dos ácidos graxos da dieta e do tecido adiposo da mama, polimorfismos e risco de doença benigna e câncer de mama**. Orientadora: Maria do Carmo Gouveia Peluzio. Coorientadores: Milene Cristine Pessoa, Helen Hermana Miranda Hermsdorff e Renata Nascimento de Freitas.

O câncer de mama (CM) é o segundo câncer mais comum entre as mulheres brasileiras, destacando-se entre os tipos de câncer com altas taxas de mortalidade. O CM é considerado uma doença multifatorial envolvendo fatores biológico-endócrinos, vida reprodutiva, comportamentais, estilo de vida, como a dieta, e os genéticos. O presente trabalho tem como objetivo investigar os hábitos alimentares, o perfil de ácidos graxos do tecido mamário e a ocorrência de polimorfismo de nucleotídeo único (SNP) entre mulheres com o CM ou doença benigna da mama (DBM). Foi conduzido um estudo caso-controle, mascarado, de base hospitalar, com mulheres diagnosticadas com CM, DBM e controles, atendidas em um hospital público da cidade de Belo Horizonte, MG. Os dados foram coletados com a aplicação de questionário, e as amostras biológicas obtidas das pacientes que foram submetidas à cirurgia da mama por meio da coleta de sangue e biopsia do tecido mamário. Os resultados mostraram que não houve diferença estatística no consumo de ácidos graxos entre os grupos avaliados. A concentração do ácido láurico (P = 0,001), ácido mirístico (P = 0,036), ácido esteárico (P = 0,031) e ácidos graxos saturados totais (AGST) (P=0,048) foram menores no tecido adiposo das mulheres com CM do que nas com DBM, enquanto o ácido palmitoleico (P = 0,022), ácido erúcico (P = 0,002), MUFAs totais) (P = 0.039) e a razão ácido oleíco/ácido esteárico (P = 0,015) foram maiores naquelas com CM do que nas com DBM. Por sua vez, não houve associação significativa entre o polimorfismo de Pro12Ala do Receptor Ativado por Proliferadores de Peroxissomos gama (*PPARγ)* (P=0,977), da Metilenotetrahidrofolato Redutase (*MTHFR)* C677T (IC=0,956-1,003), da Metionina Sintetase (*MTH)* A2756G (IC=0,335-1,028) e os grupos estudados. Quando testados se as combinações de alelos de risco poderiam gerar um efeito cumulativo no câncer de mama, o presente trabalho não encontrou associações significativas (p>0,05). As variáveis apontadas como fatores de risco para a ocorrência de DBM e CM nesta população estavam relacionadas à vida reprodutiva e hormonal da mulher, bem como

a composição corporal e de estilo de vida. A participação das variáveis principais deste estudo e o CM e a DBM, continuam dependente de mais estudos na população brasileira, particularmente em relação aos polimorfismos.

ABSTRACT

CONCEIÇÃO, Lisiane Lopes da, D.Sc., Universidade Federal de Viçosa, November, 2016. **Profile of fatty acids of diet and adipose tissue of breast, polymorphisms and risk of benign disease and breast cancer.** Adviser: Maria do Carmo Gouveia Peluzio. Co-advisers: Milene Cristine Pessoa, Helen Hermana Miranda Hermsdorff and Renata Nascimento de Freitas.

Breast cancer (BC) is the second most common cancer among Brazilian women, highlighting among cancers with high mortality rates. BC is considered a multifactorial disease involving biological-endocrine factors, reproductive life, behavioral, lifestyle, diet, and genetic. The present study aims to investigate the eating habits, the fatty acid profile of the breast tissue and an occurrence of single nucleotide polymorphism (SNP) among women with BC or benign breast disease (BBD). A masked, hospital-based case-control study was conducted with women diagnosed with BC, BBD and controls, attended at a public hospital in the city of Belo Horizonte, MG. The data were collected with the application of a questionnaire, and the biological samples obtained from patients who underwent breast surgery through blood collection and breast tissue biopsy. The results showed that there was no statistical difference in the consumption of fatty acids between the evaluated groups. The concentration of lauric acid (P = 0.001), myristic acid (P = 0.036), stearic acid (P = 0.031) and total saturated fatty acids (SFAs) (P = 0.048) were lower in women with BC than in those with BBD, whereas palmitoleic acid (P = 0.022), erucic acid (P = 0.002), total MUFAs (P = 0.039) and oleic acid / stearic acid ratio (P = 0.015) were higher in those with BC than in BBD. On the other hand, there was no significant association between Pro12Ala polymorphism of the Receptor Activated by Peroxisome Proliferators gamma (*PPARγ*) (P = 0.977), Methylenetetrahydrofolate Reductase (*MTHFR*) C677T (CI = 0.956-1.003), Methionine Synthase *(MTH*) A2756G (CI = 0.335-1.028) and the groups studied. When tested whether combinations of risk alleles could generate a cumulative effect on breast cancer, our work found no significant associations. The variables identified as risk factors for the occurrence of DBM and CM in this population were related to women's reproductive and hormonal life, as well as body composition and lifestyle. The participation of the main variables of this study and CM and DBM, remain dependent on more studies in the Brazilian population, particularly in relation to the polymorphisms.

1.INTRODUÇÃO GERAL

Mundialmente, o câncer de mama (CM) é o segundo câncer mais incidente e mais frequente entre as mulheres (1). No Brasil, a estimativa para o biênio 2016-2017, é de 57.960 novos casos desta neoplasia, reforçando, portanto, a sua magnitude no cenário nacional, que a considera um problema de saúde pública na atualidade (2). Assim, o câncer de mama é um dos principais desafios enfrentados pelo governo brasileiro, cuja taxa de mortalidade tem aumentado progressivamente nos últimos anos (3).

Estudos de coorte têm demonstrado maior risco de desenvolvimento de câncer de mama em mulheres que já tiveram a doença benigna da mama (4, 5). Recente metanálise multiétnica, revelou que a doença benigna da mama parece ser um fator de risco no desenvolvimento do câncer de mama nas mulheres avaliadas (6).

Contudo, vários são os fatores de risco reconhecidos para o câncer de mama, dentre os quais pode-se destacar os relacionados à vida reprodutiva e clínica da mulher, a idade, aos fatores genéticos e endócrinos, além do estilo de vida (7, 8). Mudanças no estilo de vida, incluindo adoção de uma dieta mais saudável, aumento da atividade física e abandono do fumo podem ter impacto positivo, pois proporcionam melhorias no modo de viver e na qualidade de vida, reduzindo, portanto, os riscos à saúde (9).

Estima-se que até 35% dos fatores de risco estejam associados a dieta (10). Estudos epidemiológicos indicam o impacto de alguns alimentos/nutrientes no risco do câncer de mama, tais como o consumo de fibras, vitaminas, minerais, frutas, hortaliças, gordura saturada e total, ácidos graxos ω-3, carnes vermelhas e processadas e álcool (10-12). No entanto, esta relação entre o consumo e o risco ainda permanece inconclusivos.

De modo geral, os mecanismos atualmente propostos, pelos quais os alimentos/nutrientes podem afetar a carcinogênese mamária, seja como fator protetor

ou desencadeador, estão relacionados à propriedade antioxidante; influência sobre processos epigenéticos, bem como no desequilíbrio das concentrações circulantes de hormônios endógenos; além de reparo do DNA; inflamação; estresse oxidativo; e também na regulação da expressão gênica (12).

Nesse contexto, os polimorfismos de nucletíodeo único (SNP - *Single Nucleotide Polymorphisms*), que são alterações na sequência de DNA de diferentes indivíduos (13), têm sido bastante estudados como fator de risco em diversos cânceres (14-16), assim como no câncer de mama (17-20) e em outras doenças crônicas não transmissíveis (21, 22). Estes polimorfismos podem resultar na produção de proteínas com funções alteradas no metabolismo humano (23), e são a fonte mais comum de variação genética em humanos (24).

Os SNP para os genes da metilenotetrahidrofolato redutase (*MTHFR*), da metionina sintetase (*MTR*) e do receptor ativado por proliferadores de peroxissomos gama (*PPARγ*) são alguns dos polimorfismos em genes importantes no câncer de mama, pois parecem modificar o risco para a ocorrência dessa doença (17-20, 25, 26).

A MTHFR, umas das enzimas chave do metabolismo de um carbono, catalisa a conversão irreversível do 5,10-metilenotetrahidrofolato em 5-metiltetrahidrofolato, sendo este o doador de metil para a síntese de metionina a partir da homocisteína (27, 28). O nível de atividade desta enzima é um fator importante no risco de desenvolvimento de alguns tipos de câncer (28). Já a MTR, é a enzima que catalisa a transferência irreversível do grupo metil da 5-metiltetrahidrofolato, promovendo a remetilação da homocisteína a metionina, sendo esta última a precursora direta da S-adenosilmetionina (29).

Os receptores ativados por proliferadores de peroxissomos (PPAR) são fatores de transcrição ativados por ligantes, que orquestram a diferenciação dos adipócitos e promovem a formação de adipócitos maduros. Atuam também como supressores de genes tumorais (30). Muitos tumores são caracterizados por alterações na homeostase lipídica com concomitantes mudanças na expressão e atividade de muitas enzimas lipolíticas e lipogênicas, sendo que muitas dessas mudanças ocorrem

em enzimas reguladas por PPAR. No câncer de mama o PPARγ está superexpresso, e apresenta efeitos antiproliferativos e proapoptóticos, podendo inibir o crescimento de tumores mamários através de um efeito direto na apoptose, no ciclo celular, na diferenciação, e na angiogênese da célula tumoral (31).

Assim, a identificação de marcadores moleculares associados ao comportamento tumoral mais agressivo e a um prognóstico ruim poderiam ser instrumentos de diagnóstico mais precoce para a escolha do tratamento mais adequado, e, por conseguinte, obter uma melhora da sobrevida das pacientes (32).

Diante do exposto, como hipótese do presente trabalho, acredita-se que as mulheres com câncer de mama possuem padrões genéticos, estilo de vida e alimentação diferentes daquelas sem a doença, havendo a necessidade de maiores investigações, a fim de se compreender os possíveis mecanismos, aprofundando nas vias relacionadas à gênese e ao desenvolvimento desta doença.

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2. OBJETIVOS DO ESTUDO

2.1 OBJETIVO GERAL

Investigar os hábitos alimentares, o perfil de ácidos graxos do tecido mamário e a ocorrência de SNP entre mulheres com o câncer de mama ou doença benigna da mama, atendidas em um hospital público de Belo Horizonte, MG.

2.2 OBJETIVOS ESPECÍFICOS

- Descrever o perfil antropométrico, socioeconômico e de saúde das mulheres em estudo;
- Avaliar o perfil de consumo de ácidos graxos;
- Comparar a composição dos ácidos graxos do tecido adiposo da mama de mulheres com câncer de mama e doença benigna da mama;
- Descrever a frequência genotípica do polimorfismo Pro12Ala do PPARγ;
- Investigar os fatores relacionados a doença benigna da mama;
- Examinar o efeito cumulativo dos polimorfismos de nucleotídeo único-SNP (C677T da MTHFR, e do A2756G da MTR) na predição da ocorrência do câncer de mama.

3. ARTIGOS

3.1 Artigo 1 (Revisão): Compostos bioativos extraídos de frutas e o desenvolvimento do câncer de mama

Introdução

Mundialmente, o câncer de mama é o segundo câncer mais comum, mais frequente entre as mulheres, ocupando a quinta posição como causa de morte, e, tal fato se deve ao prognóstico relativamente favorável da doença (1). Estima-se que um terço dos cânceres podem ser evitado por mudanças no estilo de vida, incluindo adoção de uma dieta mais saudável, aumento da atividade física e abandono do fumo (2).

As escolhas alimentares durante adolescência podem ser particularmente importantes no risco futuro de desenvolvimento do câncer de mama. Maior consumo de maçã, banana, e uvas durante a adolescência e laranjas e couve durante o início da idade adulta foram significativamente associados ao um risco reduzido de câncer de mama (3).

Em estudo com mulheres na pós-menopausa, o maior consumo de *berry*, como morangos, mirtilos e pêssegos/nectarinas foram associados ao menor risco de câncer de mama nessa população (4).

Assim, o potencial das frutas e hortaliças na prevenção do câncer de mama tem se destacado nos últimos anos. Contudo, ressalta-se que a composição das frutas e hortaliças é variável e, consequentemente, podem apresentar resultados diferentes quando do uso na prevenção ou mesmo tratamento da doença (4).

Ademais, como o câncer é uma doença multifatorial, a prevenção e o tratamento ideais no câncer de mama devem atingir múltiplas vias bioquímicas e moleculares. Desta forma, raramente, terapias que utilizam um único agente como alvo tem efeito positivo na prevenção ou mesmo na cura dos pacientes com câncer (5).

Considerando o atual debate no meio científico em relação aos alimentos que contêm substâncias com o potencial de inibir, retardar ou reverter a carcinogênese, o presente artigo teve como objetivo revisar o conhecimento sobre o potencial benéfico das frutas no processo de desenvolvimento do câncer de mama.

Berries

A inclusão das *berries* nas refeições tem um impacto positivo na resposta glicêmica pós-prandial, melhora o perfil de marcadores inflamatórios e aumenta a capacidade antioxidante em humanos. Somado a isto, o alto teor de compostos fenólicos das *berries* atuam suprimindo a invasão patogênica nas células epiteliais intestinais, contribuindo na promoção da saúde intestinal por modificar o microbioma (6).

De modo geral, os fitoquímicos derivados das *berries* são conhecidos como agentes quimiopreventivos, atuando no combate à oxidação, à radiação ultravioleta, aos danos ao DNA, à infecção bacteriana bem como atuando em cascatas de sinalização celular, mecanismos pró-inflamatórios, no crescimento e proliferação de células carcinogênicas (7).

Os componentes bioativos das *berries* mais estudados são as antocianidinas, proantocianidinas, flavonóis, estilbenoides, terpenóides, elagitaninos e ácido elágico (7).

Em um estudo com diferentes linhagens celulares malignas da mama humana, estas células foram tratadas com extratos hidroalcoólicos da casca, da semente e do açaí total nas concentrações de 10, 20 e 40 μg/mL por 24 e 48 h. Apenas a linhagem celular de câncer de mama humano estrogênio dependente MCF-7 respondeu ao tratamento com o açaí, verificando-se redução da viabilidade celular, alterações morfológicas e indução de autofagia. O tratamento com extrato da semente e do fruto total foram mais eficaz do que o extrato da casca (8).

Purple berries (*Eugenia jambolana* Lam.) é proveniente de uma planta indiana nativa, que quando maduros, seus frutos comestíveis são conhecidos como

Jamelão (Brasil), Jamun ou Jambul (Índia), Jamu plum (Estados Unidos) e se assemelham na aparência a uma uva roxa. Os efeitos anti-proliferativos e pró-apoptóticos do extrato desta *Berry*, rico em antocianinas, foi testado *in vitro* em células de câncer de mama (estrogênio dependente/aromatase positiva - MCF-7aro e estrogênio independente - MDA-MB-231) e também em células normais da mama (MCF-10A) em concentrações variando de 0 - 100 μg/mL, em três momentos diferentes de 24, 48 e 72 h e 100 e 200 μg/mL por 24 h, respectivamente. Interessantemente, a redução na proliferação celular foi dose e tempo dependente, e, mais efetivo na linhagem celular MCF-7aro quando comparado a MDA-MB-231. Nas células normais da mama (MCF-10A), o extrato exibiu apenas efeitos antiproliferativos suaves, sugerindo, portanto, sua ação específica sobre as linhagens celulares tumorigênicas, indicando que o extrato não é tóxico em linhagens celulares não transformadas (9).

O efeito quimiopreventivo do extrato da *blueberry* foi avaliado *in vitro* e *in vivo*, em modelo xenotransplantado MDA-MB-23. De modo similar, ao estudo com *Purple berries* descrito anteriormente, o extrato de *blueberry* apresentou efeito antiproliferativo nas linhagens celulares tumorigênicas mamárias testadas (HCC1937 e MDA-MB-231) sem efeito sobre a linhagem celular não tumorigênica (MCF-10A) (10). O extrato alcoólico de *blueberry* inibiu fortemente também as linhagens de câncer de mama MCF-7 e T47-D (11).

In vivo, a ingestão de *blueberry* em ratos diminuiu não apenas o crescimento e proliferação tumoral, mas também a atividade da serina/ treonina cinase 1 (AKT) e fator nuclear kappa B (FN-kB), que são marcadores potenciais de metástase em tumores de mama, e, verificou-se, ainda, o aumento da apoptose. Os autores sugerem que o efeito inibidor dos fitoquímicos da *blueberry* ocorram através da modulação da via de sinalização PI3K/AKT/FN-κB (10).

Extratos de seis *berries* consumidas popularmente, amora, framboesa preta, mirtilo, oxicoco, framboesa vermelha e morango foram testadas em cultura de células de câncer de mama humano (MCF-7), em concentrações variando de 25 a 200 micro g/mL. Os resultados revelaram que com o aumento da concentração do

extrato das *berries* testadas, foi observada uma crescente inibição da proliferação celular nesta linhagem celular (12).

Extrato aquoso de framboesa e do morango obtido do fruto liofilizado foram capazes de inibir a mutagênese em culturas de células de câncer da mama, MCF-7 e T47-D (11).

Extrato de morango foi testado durante 24 h, 48 h ou 72 h com concentrações variando de 0,5 a 5 mg/ml em uma linhagem celular de câncer de mama altamente agressiva e invasiva (A17), e verificou-se que o extrato foi capaz de reduzir fortemente a viabilidade celular, de maneira dose e tempo dependente. Outras culturas de células tanto cancinogênicas quanto normais também foram avaliadas e observou-se que o extrato foi mais efetivo no combate a sobrevivência celular nas células de câncer de mama. Foram verificadas também alterações na distribuição das fases do ciclo celular e na motilidade bem como modulação de genes envolvidos em processos de migração celular, adesão e invasão (13).

Experimentação *in vivo* em ratos que receberam 15% de extrato de morango (*Fragaria* x *ananassa*) na dieta revelaram redução significativa no peso e volume do tumor, sugerindo um potencial anti-invasivo interessante deste extrato (13).

Maçã

A maçã é o fruto pomáceo da macieira, arvóre da família Rosaceae, gênero Malus, englobando aproximadamente 25 espécies. É boa fonte de polifenoís e de fibra alimentar, especialmente a pectina, além de apresentar interessante atividade antioxidante (14).

Estudo realizado com o extrato de maçã, numa dose de 5 mg/mL, inibiram significativamente a ativação do NF-kB em culturas de células de câncer de mama humano MCF-7. O NF-kB induz resistência a agentes quimioterapêuticos anticâncer, aumentando a proliferação celular e inibindo a apoptose, nas células cancerosas (15). Desta forma, o extrato da maça apresentou um efeito benéfico na terapia do câncer de mama por reduzir a resistência à quimioterapia.

Por outro lado, efeito sinérgico na inibição da proliferação das células MCF-7 foi observada quando o extrato de maça foi combinado com a quercetina, ou seja, a combinação de ambos potencializou a atividade antiproliferativa (16).

Melão

O melão amargo (*Momordica charantia*) originado na Ásia Tropical tem demonstrado atividade anticâncer em estudos *in vitro* e *in vivo*. O óleo da semente do melão amargo é rico em ácido eleosteárico, um ácido graxo poli-insaturado de cadeia longa, cujo efeito foi investigado tanto em culturas de células de câncer de mama humano com receptor de estrogênio negativo (MDA-MB-231) quanto positivo (MDA-ERα7). Até o momento, o ácido eleosteárico parece ser capaz de bloquear a proliferação celular do câncer de mama e induzir apoptose (17). Neste contexto, estudo avaliou o efeito do extrato do melão amargo em três diferentes concentrações (1%, 2% e 5%, v/v) em culturas de células de câncer de mama humana, MCF-7 e MDA-MB-231, e também nas células epiteliais mamárias humanas primárias. O tratamento com o extrato induziu a morte celular destas linhagens de carcinoma mamário humano bem como a inibição do crescimento celular (18). Ainda em culturas de células de câncer de mama humano, MCF-7 e MDA-MB-231, um composto isolado do *Momordica charantia* L. e muito usado como fitofármaco, o triterpenóide de cucurbitano, induziu a morte apoptótica e a autofagia das células de câncer de mama avaliadas (19).

Em experimentação animal, com camundongos SHN, com livre acesso ao extrato de melão amargo (0,5%) em água potável, foi verificado uma inibição significativa do desenvolvimento dos tumores mamários (20).

Limão

Entre as árvores frutíferas, o gênero *Citrus* é a mais importante no mundo em termos de colheita, e o limão considerado a terceira espécie cítrica mais importante.

Neste sentido, o limão tem tido destaque na promoção da saúde, uma vez que é rico em compostos fenólicos, bem como vitaminas, minerais, fibras alimentares, óleos essenciais e carotenoides (21).

Limonóides extraídos da semente do limão (*Citrus lemon* L. Burm) apresentaram citotoxicidade em linhagens de câncer de mama humanos MCF-7 e MDA-MB-231. A inibição na proliferação celular foi significativamente correlacionada com a ativação da caspase-7, envolvida no processo de apoptose. Contudo, a atividade antiproliferativa observada não foi relacionada com a atividade anti-aromatase (22).

Uva

A uva é o fruto da videira (Vitis sp.), uma planta da família das *Vitaceae*. Sementes e peles de uva são boas fontes de fitoquímicos, como ácido gálico, catequina e epicatequina, compostos estes com capacidade antioxidante. O extrato da semente da uva possui alto teor de antioxidantes na forma de proantocianidinas. A capacidade antioxidante deste extrato tem sido relatada como maior do que a observada para as vitaminas C e E. O consumo regular tanto dos extratos da semente da uva quantos os produtos à base de uva parecem ser benéficos para a população em geral (23).

As proantocianidinas da semente de uva foram avaliadas quanto ao seu efeito no mimetismo vasculogênico (MV), mecanismo no qual as células tumorais são capazes de formar canais vasculares estruturados rodeados pelas células tumorais sem a participação das células endoteliais e independente da angiogênese. Este processo é considerado um grande obstáculo na resistência à terapia com drogas anti-angiogênicas. E, no contexto do câncer de mama, este processo é ainda mais relevante uma vez que o mesmo é classificado como um tumor muito vascularizado (24).

Neste sentido, o tratamento com as proantocianidinas da semente de uva em variadas concentrações (0,50 μg / ml, 100 μg / ml, 200 ug / ml) por 24 e 48h em

cultura de célula humana de câncer de mama HCC1937 demonstrou os seguintes efeitos: inibição na expressão da proteína Twist1, envolvida na metástase; inibição do crescimento tumoral e dos canais MV bem como perda de marcadores epiteliais, E-caderina, e a ativação de marcadores mesenquimais, VE-caderina. Tal transição epitelial-mesenquimal é uma etapa chave no processo de metástase tumoral (24).

O extrato da semente de uva em alta concentração (100 µg/ml) inibiu a proliferação celular e a apoptose nas linhagens celulares MDA-MB231 e MCF-7, ao passo que em baixa concentração (25 µg/ml) foi capaz de reduzir a migração e invasão celular (25).

Estudos com resveratrol, um polifenol encontrado na pele de uva, vinho, uva inteira, demonstraram atividades anticâncer em culturas de células MFC-7, induzindo a autofagia (26) e inibindo a formação de adutos de DNA (27, 28).

Romã

A romã (*Punica granatum*) apresenta uma gama de aplicações no câncer observadas em diferentes compartimentos do fruto. O suco e a casca possuem propriedades antioxidantes enquanto que o suco, a casca e o óleo demonstraram atividades anticancerígenas, interferindo na proliferação de células tumorais, ciclo celular, invasão e angiogênese (29).

O polifenóis da romã (*Punica granatum*) avaliados em suco fermentado, extrato aquoso do pericarpo e em óleo da semente, se mostraram eficaz contra o câncer da mama através da inibição da atividade da aromatase, ou seja, via bloqueio da biossíntese endógena de estrogênio, bem como na inibição da proliferação das linhagens celulares tumorigênicas avaliadas (MCF-7 e MB-MDA-231) (30). Outro estudo avaliou o efeito do suco de romã nestas mesmas linhagens, MCF-7 e MB-MDA-231, e a inibição do crescimento também foi verificada, além da diminuição na migração de células carcinogênicas, porém estes efeitos encontrados não afetaram as células normais (MCF10A) (31).

De modo similiar, o extrato da romã inibiu o crescimento das linhagens celulares de carcinoma mamário testadas (BT-474 e MDA-MB-231), mas não as não-carcinogênicas (MCF-10F e MCF-12F), e diminuiu também o volume tumoral. A citotoxidade induzida pelo extrato da romã foi acompanhada pela ativação da caspase-3, enzima primária na execução da apoptose. O tratamento reduziu significativamente os fatores de transcrição de Sp (Sp 1, Sp2, Sp3), envolvidos na regulação de genes necessários para a sobrevivência celular e angiogênese, o que resulta na inibição do crescimento e na morte celular apoptótica (32).

Experimentação *in vivo*, em ratos com tumorigênese mamária induzida pelo composto químico cancerígeno (DMBA) e tratados com uma emulsão de romã (formulação patenteada composta por extrato de fase aquosa de romã e o óleo da semente), observou-se inibição da tumorigênese mamária nas concentrações avaliadas (0,2, 1,0 e 5,0 g/kg), de maneira dose-resposta. O tratamento também reduziu a expressão intratumoral do receptor de estrogênio alfa (ER-α), receptor de estrogênio beta (ER-β) e da β-catenina bem como inibiu a expressão da proteína reguladora do crescimento celular, a ciclina D1 (33).

Uma fração considerável dos canceres de mama é estrogênio dependente, o uso de componentes da dieta capazes de atuar como inibidores da aromatase, suprimindo a formação de estrogênio, e também na modulação dos receptores de estrogênio alfa e beta, poderiam auxiliar na supressão da carcinogênese da glândula mamária. O uso de fármacos antiestrogênicos capazes de competir com o estrogênio na ligação aos em seus receptores, é conhecida por ser eficaz para impedir o crescimento de tumores mamários dependentes de estrogênio, bem como para prevenir a ocorrência de tumor de mama (9, 33).

Ameixa

A ameixa (*Achras sapota*) pertence à família *Sapotacea*, e apresenta atividade antioxidante interessante (34). O extrato da ameixa (*Achras sapota*) foi capaz de

induzir a citotoxicidade na cultura de células de câncer de mama humano, MCF7, através da ativação da via de apoptose, de maneira dose dependente.

Também em culturas MCF-7, extratos de três variedades de ameixa (Byron gold, Black Splendor, e Burgundy) exibiram atividades anti-proliferativa e pró-apoptóticas, sendo que efeitos pequenos ou nulos foram observados na linhagem de células de mama não cancerosas MCF-10A (35).

Na experimentação *in vivo*, o tratamento com o extrato inibiu a progressão tumoral, resultando num aumento da sobrevida em cerca de 50%. Os autores deste estudo concluiram que a inclusão desta variedade de ameixa na dieta diária pode proteger da gênese e progressão do câncer (36).

Fruta do dragão (Pitaia)

Fruta conhecida no Brasil como Pitaia e na Ásia como Fruta do Dragão, é uma fruta rústica, pertencente à família *Cactaceae*. No cerrado brasileiro, é comum de serem encontradas as denominadas pitaya-do-cerrado, pitaya-vermelha ou saborosa (37).

O extrato da casca de duas variedades da fruta do dragão, *H. polyrhizus* e *H. undatus*, exibiram atividades antioxidantes e citotóxicas na linhagem celular de câncer de mama (Bcap-37). O efeito inibitório na proliferação celular de *H. polyrhizus* foi mais forte do que o de *H. undatus.* Os dois extratos avaliados mostraram atividade antioxidante, dependente da dose (38).

Considerações Finais

A indução, o crescimento e a progressão do câncer são eventos de múltiplos passos e os estudos desenvolvidos até o momento demonstraram que vários agentes da dieta interferem nestes estágios, bloqueando ou mesmo diminuindo a progressão e a malignidade da doença.

Vários são os compostos bioativos presentes nas frutas, derivados de diferentes estruturas químicas, incluindo fenólicos (taninos, lignanas, flavonóides), glucosinolatos, terpenóides, carotenóides e fitoestrógenos, que apresentam potencial anticancerígeno ou eficácia quimiopreventiva.

Os efeitos dos compostos bioativos extraídos das frutas incluídas neste artigo revelam dados positivos. De modo geral, os mecanismos mais destacados foram: indução da apoptose celular; inibição da proliferação celular; modulação de vias de sinalização importantes no câncer de mama, tais como PI3K/ AKT/ FN-κB bem como na expressão de marcadores envolvidos na cascata de sinalização do câncer de mama, como os epiteliais, os reguladores do crescimento celular, os receptores de estrogênio α e β, os envolvidos no processo de metástase e outros. Devemos ter em mente que os resultados aqui apresentados em sua maioria derivam de estudos *in vitro* e tem-se como limitação o fato de não poder extrapolar os dados encontrados para os humanos em tratamento.

Estes compostos bioativos geram muito interesse no meio científico, porque cumprem os requisitos básicos de um agente quimiopreventivo ideal, tais como toxicidade seletiva para células cancerosas ou precancerosas, eficácia contra a maioria dos tipos de câncer, além da administração via oral e boa aceitação.

O potencial quimiopreventivo dos agentes naturais presentes nas frutas, incluídas na dieta ou em outros procedimentos discutidos nesta revisão, devem ser avaliados em estudos *in vivo*, visando o conhecimento e controle quanto à toxicidade, em paralelo aos estudos observacionais com o intuito de se ter informações do consumo habitual ou mesmo das aplicações populares, e por fim estudos clínicos com humanos para certificar se os efeitos observados na fase pré-clínica de fato ocorreriam. Em caso positivo, estaremos diante de estratégias alternativas eficazes para o tratamento do câncer, seja administrado isoladamente ou em combinação com drogas anticâncer já disponíveis no mercado, em função da atuação destes componentes bioativos em múltiplos efeitos biológicos, como alvos e vias de sinalização.

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3.2 Artigo 2 (Original): Difference in fatty acids composition of breast adipose tissue in women with breast cancer and benign breast disease.

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Abstract

Introduction: Breast cancer is the second most common cancer in the world, and the most frequent cancer among women. Moreover, there are factors that influence the risk for breast cancer including the age, genetic and endocrine factors, and lifestyle.

Objectives: Evaluate the consumption of fatty acids; compare the fatty acids composition in the breast adipose tissue of women with breast cancer and benign breast disease as well as potential risk factors; and describe the genotypic frequency of the Pro12Ala PPARγ polymorphism.

Material and Methods: A hospital-based case-control study was conducted including incident cases (*n* = 38 breast cancer; *n* = 75 benign breast disease; *n* = 166 control). Lifestyle features, socioeconomic issues, dietary intake, anthropometry, and blood and tissue data were assessed.

Results: No differences were observed for fatty acids intake. Interestingly, lauric acid (*P* = 0.001), myristic acid (*P* = 0.036), stearic acid (*P* = 0.031), and total saturated fatty acids (SFAs) (*P*=0.048) had lower concentrations in BC than in BBD women, while palmitoleic acid (*P* = 0.022), erucic acid (*P* = 0.002), total monounsaturated fatty acids (MUFAs) (*P* = 0.039) and oleic acid/stearic acid ratio (*P* = 0.015) increased. There was no significant association between PPARγ polymorphism and studied groups (*P* = 0.977). The age at first full pregnancy (*P* = 0.004) was significantly associated with the development BC, whereas BMI (*P* =

0.005); percentage of body fat (P = 0.024); physical activity (P = 0.036); and age at menarche (P = 0.008), at first full pregnancy (P < 0.001), and of first mammogram (P = 0.018) were significantly associated with the development of BBD.

Conclusion: The results suggest a different fatty acids composition of breast adipose tissue, a biomarker of long-term dietary intake, particularly for SFAs, MUFA and 18: 1 n-9/18: 00 ratio. Our findings also show that are differences in the factors related to the development of BC and BBC.

Keywords: Fatty acids; breast cancer; benign breast disease; PPARγ; dietary intake.

ABBREVIATIONS

BC: Breast cancer

FA: Fatty acid

PPARγ: Peroxisome proliferator-activated receptor gamma

BBD: Benign breast disease

C: Control group

SFFQ: Semiquantitative food frequency questionnaire

PAQ: International physical activity questionnaire

WHR: Waist to hip ratio

WHtR: Waist to height ratio

BMI: Body mass index

SFA: Saturated fatty acids

MUFA: Total monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

SCD1: Enzyme stearoyl-CoA desaturase-1

Introduction

Breast cancer (BC) is the second most common cancer in the world, and the most frequent cancer among women [1]. There are several recognized risk factors for BC, mainly age, genetic and endocrine factors, and lifestyle [2]. It is estimated that up to 35% of risk factors are associated with diet [3].

However, the assessment of the association of diet components with BC risk is not an easy task because of the limitations of conventional methods to assess dietary intake, such as memory, difficulty in estimating portion size, day-to-day variability, seasonal eating patterns, and use of the food consumption tables [4, 5]. In fact, the determination of tissue nutrients may provide a more accurate estimate of dietary intake. The fatty acid (FA) content of the adipose tissue has been proposed as a biomarker of FA intake most appropriate because it reflects the long-term ingestion, up to 2 years previous, when no severe weight loss had occurred [6].

The peroxisome proliferator-activated receptor gamma (PPARγ) has been shown to be important in many biochemical functions such as the adipocyte differentiation and also act as a tumor suppressor gene, inhibiting the growth of several cell types, and induction of apoptosis [7]. However, until this moment, studies investigating the association between PPARγ polymorphism and the risk of BC reported inconclusive results [8-14].

Overall, the aims of this study were to evaluate the consumption of fatty acids; to compare the fatty acids composition of the breast adipose tissue of women with BC and benign breast disease (BBD); to identify factors associated with risk of developing of BC and BBD; as well as to describe the genotypic frequency of PPARγ Pro12Ala polymorphism.

Materials and Methods

Sample Study

This is an double-blind, hospital-based, case-control study conducted with women attending the mastology and/or gynecology service of a public hospital in Belo Horizonte, Brazil. All women attended between January and July 2006 were

invited to participate in the study. In this study, we included only women without previous diagnosis of BC or BBD. The volunteers were divided in three groups: case, women with histological diagnosis of malignant breast disease; BBD, women diagnosed with fibrocystic breast changes or other non-proliferative BBD; and Control (C) women who underwent a routine examination or gynecological surgery and had a recent mammogram result. The final sample was composed of 229 women. Written consent was given by all women after they had been informed of the objective and protocol of the study. The study followed principles of the Declaration of Helsinki and was approved by the National Committee of Ethics in Research (protocol number: 1889/2005).

Data Collection

Information about lifestyle, as well as gynecological and obstetric history, and socioeconomic issues were collected using a previously validated questionnaire for the population of the region studied [15]. Dietary intake was assessed using a semiquantitative food frequency questionnaire (SFFQ).

Volunteers who consumed at least 1 dose (10 g of alcohol) of any alcoholic beverage/day or in a frequency of more than 3 days/week were considered alcoholic [16]. In the same way, who smoked at least 1 cigarette per day, regardless of the time of use were considered smokers. Physical activity was assessed using the short version of international physical activity questionnaire (IPAQ) [17].

Anthropometric measurements such as weight, height, waist, and hip circumference were obtained from all the participants according to the standard protocol [18-20]. The waist to hip ratio (WHR), waist to height ratio (WHtR), and body mass index (BMI in kg/m^2) were calculated. Overweight and obesity were defined as BMI $\geq$25.0 to 29.9 kg/m^2 and $\geq$29.9 kg/m^2, respectively [21].

Moreover, the total body fat (%) was estimated by bioelectrical impedance vertical Tanita® (ModelTBF 531, Tanina Corporation of America, Illinois, USA) and classified by Gallagher et al. [22].

The collection of biological material occurred on the day of surgery after 12 h fasting. Blood and breast adipose tissue samples were collected and immediately protected from light and stored in liquid nitrogen at −80 °C until the time of analysis.

Fatty acids in breast adipose tissue

The lipids of the breast adipose tissue were extracted by Folch [23] methodology and saponified and esterified according to Hartmann and Lago [24]. The FA methyl esters were identified by gas chromatography (CG-17A Shimadzu®/Class model) [25]. Peak identification was made by comparison of their retention times with that of a mixture of commercial standards (FAME mix, Supelco®, USA). FA composition was expressed as percentage of the lipid fraction relative to the total FA content of the sample.

Genotyping

Genomic DNA was obtained from stored buffy coat. Briefly, buffy coats were digested using lysing solution, Madissen (0.1M Tris-HCl pH = 8.0; 0.4 M EDTA; 0.2% SDS; 1M NaCl; pH = 8.0), followed by addition of proteinase K (20 mg/mL) and incubated overnight at 37 °C. Then, the DNA was precipitated with saturated phenol and chloroform:isoamyl alcohol (24:1). Finally, cold isopropyl alcohol was added and homogenized slowly until the precipitation of DNA, which was dried at room temperature.

To detect the presence the proline 12 alanine (Pro12Ala) polymorphism, a 257-bp fragment of the PPARγ gene was selectively amplified by PCR [26]. The amplified fragment was digested with the restriction enzyme BstU-I according to manufacturer's instructions (Promega®, Madison, WI, USA), and the products of digestion were analyzed in polyacrylamide gel. The genotyping of patients were determined as follows: a single 257 bp fragment for the CC (Ala12Ala) genotype; two fragments of 223 and 34 bp for the GG (Pro12Pro) genotype; and three fragments of 257, 223, and 34 bp for the CG (Pro12Ala) genotype.

Statistical Analyses

Normal distribution of data was determined by Kolmogorov–Smirnov test. The Kruskal–Wallis and analysis of variance (ANOVA) was used to determine the differences in median and mean values, respectively, between the BC, BBD, and C groups. Associations between categorical variables were tested by Pearson's chi-squared test, and when necessary the chi-square partition test with Bonferroni correction was utilized. Odds Ratio and 95% confidence intervals for risk of BC and BBD were examined using multinomial logistic regression. Initially, we applied simple multinomial logistic regression, and the independent variables with significance <0.20 were considered as candidates for the final model. Then, multiple multinomial logistic regression was conducted in which the variables remained with a final model with significance level of $\alpha \leq 0.05$.

The food consumption data were log-transformed before statistical analyses, and the data were adjusted by energy according to the residual model [27].

Hardy–Weinberg Equilibrium was tested to compare the observed with expected genotype frequencies. Frequencies of the genotypes of PPARγ polymorphism between the study groups was performed by the chi-square test. All analyses were conducted in SPSS® software, version 20.

Results

The anthropometric, clinical, sociodemographic and lifestyle characteristics of BC, BBD, and C are described in Table 1. As it can be seen, the median age was higher in BC group ($P < 0.001$), and they made less use of oral contraceptives ($P = 0.011$). Furthermore, when compared to BBD group, women with BC had made their first mammogram ($P < 0.001$) at a later age and presented menopause at an older age too ($P = 0.037$) suggesting a longer interval between ages at menarche and natural menopause.

However, in control group, women had the first full pregnancy younger ($P < 0.001$). While women without the disease had breastfed more ($P < 0.001$), had no

family history of breast cancer, ($P < 0.001$), or previous history of benign breast lesion ($P < 0.001$). The others parameters did not differ between the study groups.

In relation to food consumption, no differences were observed between the three evaluated groups (Table 2). However, the composition of fatty acids from the breast adipose tissue was different between groups (Table 3). The tissue concentration of lauric acid, myristic acid, stearic acid, and total saturated fatty acids (SFAs) were lower in BC than in BBC, while palmitoleic acid, erucic acid, total MUFAs, and oleic acid/stearic acid ratio were higher in BC than in BBC, reinforcing the hypothesis regarding the necessity of balance in consumption of fatty acids in dietary lipids.

The observed frequency of genotypes was not different from the expected frequency demonstrating to be in Hardy–Weinberg equilibrium in this population. Moreover, no significant association was observed between PPARγ Pro12Ala polymorphism and studied groups (p=0.977). The percentage of women with the genotype CG or GG was 17.4, 30.4, and 52.2%, in the BC, BBD, and C groups, respectively.

About the risk factors, it was observed that the age at first full pregnancy ($P = 0.004$) was highlighted in this study as an important factor associated with the development of BC. In addition, for women with BBD, BMI ($P = 0.005$), total body fat ($P = 0.024$), physical activity ($P = 0.036$), and age at menarche ($P = 0.008$), at first full pregnancy ($P < 0.001$), and of first mammogram ($P = 0.018$) were pointed as risk factors for the occurrence of BBD (Table 4).

Discussion

Great efforts have been made in the scientific community to improve our understanding of the factors associated with breast cancer [1]. However, few studies assessed the BBD and its risk factors as well the behavior of this disease.

We found that women with BC had the menopause in older age. Recent study reported that women with the longest reproductive lifespan were 1.5–1.7 times more likely to have BC compared with women with the shortest reproductive lifespan [28].

However, in control group, women had the first full pregnancy at younger age, which is according to recent evidence to suggest that pregnancy at an early age has a strong protective effect against BC in humans, through changes in hormonal dynamics and pronounced changes in gene expression [29]. In addition, in our study, the oral contraceptive use demonstrated a protective action, different from what has been described in the literature [30, 31].

To our knowledge, this is the first case-control study that utilized WHtR as screening tool, and we found the difference between groups and WHtR. Recent evidence suggests that WHtR is a better measure of the health risk such as obesity and cardio-metabolic risk factors, wherein the larger the ratio the greater the risk.

In relation to the profile of fatty acid determined in patients with CA and DBM, our results were contrary to that described in the literature, and this can be possibly due to characteristics significantly different (Table 1). Contrary to our results, concentrations of myristic acid were elevated in cancer breast tissue in a Greek women [32]. Greek patients with BC had significantly higher total MUFA ($P < 0.001$), lower total SFA ($P < 0.01$) in breast adipose tissue compared to patients with benign breast tumors, which is consistent with the present study [33]. In addition, we suggest that the differences observed in this analysis of FA as biomarker intake (Table 3), may occur due to differences in long-term food intake.

Our finding of a higher oleic acid/stearic acid ratio ($P = 0.015$) in BC women, also be attributed to the novel functions of enzyme stearoyl-CoA desaturase-1 (SCD1), related to cancer and possibly this enzyme may be overexpressed and highly active in women with BC in population. The SCD1 is a key regulator of lipid FA composition in mammalian cells and also responsible for the conversion of stearic acid to oleic acid. However, novel functions have been proposed to this enzyme like modulation of metabolic and signaling processes related to cell proliferation, survival, and malignant transformation to cancer. Thereby, has been proposed a relationship between SCD1 activity and tumor growth. In several types of cancers, elevated SCD1 expression and activity have been detected [34].

Even in relation to the polymorphism, others studies also found no association between PPARγ polymorphism and the risk of BC in different populations such as: Caucasian women [12]; Mexican women [14]; women living in Hawaii and California recruited in a Multiethnic Cohort study [13]. These reinforce that the results are still inconclusive.

In this study, only age at first full pregnancy was significantly associated with the development BC, whereas BMI, total body fat, physical activity, and age at menarche, at first full pregnancy and of first mammogram associated of BBC. Epidemiological evidences show that there are factors associated with an increased risk of BC, such as gender being a woman is the strongest risk factor for BC, increasing age, younger age at menarche, and family history. Other factors are associated with a decreased risk, such as earlier age at first birth, breastfeeding, parity, and physical activity [35].

Conclusion

The findings of the present study strengthen the hypothesis that women with BC and BBD have different sociodemographic, anthropometric, reproductive, gynecological, and lifestyle characteristics. In addition, significant associations between specific breast tissue SFAs, MUFAs, and 18:1 n-9/18:0 ratio were observed and can be supported by a physiological mechanism involving the enzyme SCD1. No association was observed between PPARγ Pro12Ala polymorphism and the studied groups, which reinforces the need for further studies since the literature shows inconclusive results on this polymorphism and breast cancer.

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Table 1: Anthropometric, clinical, sociodemographic, and lifestyle characteristics of study participants, by groups.

Variables	Groups			p
	BC (38)	BBD (75)	C (116)	
Age (years)	53 ± 26[a]	43 ± 22[b]	47 ± 10[c]	<0.001[*♦]
BMI (kg/m^2)	27.51 ± 6.50[a]	24.48 ± 4.64[b]	27.33 ± 5.40[a]	0.001[**▪]
WHR	0.88 ± 0.08	0.85 ± 0.06	0.87 ± 0.07	0.058[**]
WhtR	0.59 ± 0.10[a]	0.52 ± 0.07[b]	0.57 ± 0.08[a]	0.001[**▪]
Total body fat (%)	33.47 ± 9.23	31.30 ± 7.93	34.16 ± 7.95	0.063[**]
Age at menarche (years)	13.00 ± 2.00	12.00 ± 3	13 ± 2	0.134[*]
Age at menopause (years)	48.9 ± 5.46[a]	44.32 ± 7.57[b]	46.66 ±5.05[ab]	0.037[**▪]
Age at first full pregnancy (years)	24.50 ± 12.00[a]	24.00 ± 8.0[a]	21.00 ± 6.00[b]	0.001[*♦]
Age of first mammogram (years)	43.0 ± 15.00[a]	39.0 ± 31[b]	40.0 ± 9.00[ab]	<0.001[*♦]
Breast feeding (yes%)	24 (63.15)[a]	36 (48.00)[a]	96 (82.75)[b]	<0.001[***♯]
Nulliparity (yes,%)	14 (36.84)[a]	28 (37.33)[a]	11 (9.48)[b]	<0.001[***♯]
Oral Contraceptive use (yes,%)	14 (36.84)[b]	48 (64)[a]	72 (62.06)[a]	0.011[***♯]
Hormone replacement therapy (yes, %)	5 (13.15)	8 (10.66)	16 (13.79)	0.814[***]
Live in countryside (yes,%)	13 (34.21)[b]	53 (70.66)[a]	74 (63.79)[a]	0.0004[***♯]
***Per capita* income (U$)**	116.66 ± 111.09[a]	140.83 ± 103.12[b]	100.00 ± 118.33[a]	0.047[*♦]
Educational level (n, %)	37	74	116	0.067[***]
Primary	26 (70.27)	40 (54.05)	86 (74.13)	
Secondary	10 (27.03)	29 (39.18)	25 (21.55)	
University/Post-graduation	1 (2.70)	5 (6.77)	5 (4.32)	
Physical activity (n, %)				0.088[***]
Sedentary	20 (52.63)	36 (48.00)	41 (35.34)	
Light	15(39.47)	24 (32.00)	56 (48.27)	
Moderate	3 (7.90)	15 (20.00)	19 (16.37)	
Smoking (yes,%)	3 (7.90)	12 (16.00)	19 (16.37)	0.407[***]
Alcool intake (g/day)	4.2 ± 13.69	3.63 ± 9.79	6.13 ± 17.86	0.218[*]
Family history of breast cancer (yes,%)	10 (26.31)[a]	13 (17.33)[a]	0[b]	<0.001[***•]

BC: breast cancer; BBD: benign breast disease; C: control group; BMI: body mass index; WHR: waist to hip ratio; WhtR: waist to height ratio [*]Kruskal-Wallis Test; [**] Oneway ANOVA; ▪ Tukey Test; ♦ Dunn Test; [***] Chi-Square Test; ♯ Chi-Square partition Test with Bonferroni correction. • Fisher exact Test with Bonferroni correction. Values expressed as mean ± standard deviation for parametric variables and median and interquartile interval for non-parametric variables. Same letters in the same row represent absence of significant difference.

Table 2: Consumption of specific fatty acids of study participants, by groups

Fatty acid intake (g/day)[a]	Groups			p*
	BC (38)	**BBD (75)**	**C (116)**	
12:0 (lauric acid)	0.27 (0.08 – 1,45)	0.29 (0.05 –1.19)	0.18 (0.02 – 0.68)	0.059
14:0 (myristic acid)	0.90 (0.38 – 4.82)	1.29 (0.34 – 3.97)	0.94 (0.27 – 2.64)	0.477
16:0 (palmitic acid)	11.89 (5.15 – 19.12)	11.65 (7.40 – 20.76)	8.91 (5.00 – 15.70)	0.326
18:2 n6 (linoleic acid)	10.15 (7.47 – 15.41)	15.08 (10.20 – 24.34)	13.24 (7.10 – 25.08)	0.304
18:3 n3 (α-linolenic acid)	1.07 (0.77 – 1.61)	1.47 (0.89 – 2.70)	1.41 (0.74 – 2.79)	0.368
20:4 n-6 (arachidonic acid)	0.03 (0.00 – 0.09)	0.03 (0.01 – 0.12)	0.02 (0.01 – 0.07)	0.439
20:5 n-3 (eicosapentaenoic acid)	0.01 (0.00 – 0.02)	0.00 (0.00 – 0.02)	0.00 (0.00 – 0.02)	0.999
22:6 n-3 (docosahexaenoic acid)	0.01 (0.00 – 0.03)	0.01 (0.00 – 0.08)	0.01 (0.00 – 0.05)	0.663
Total SFAs	27.95 (11.16 – 44.45)	29.39 (18.50 – 59.23)	24.45 (12.53 – 39.60)	0.208
Total MUFAs	19.74 (12.57 – 39.12)	33.77 (18.25 – 62.24)	46.41 (14.41 – 49.55)	0.069
Total n-6 PUFAs	10.18 (7.49 – 15.41)	15.31 (10.20 – 24.40)	13.28 (7.22 – 25.16)	0.301
Total n-3 PUFAs	1.07 (0.76 – 1.59)	1.59 (0.92 – 2.66)	1.42 (0.76 – 2.75)	0.324
Total PUFAs	16.21 (10.85 – 26.37)	23.51 (14.87 – 35.93)	22.38 ± 25.98 (12.86 – 38.83)	0.281
Total Lipids	77.60 (41.63 – 122.97)	87.69 (66.74 – 178.25)	73.62 ± 86.84 (43.73 – 130.56)	0.254

BC: breast cancer; BBD: benign breast disease; C: control group; *ANOVA Test; [a] Fatty acid intake was adjusted by energy intake using residual model. Values are median (25th-75th quartile).

Table 3: Breast adipose tissue concentration of fatty acids in breast cancer and breast benign disease women.

Variables	Groups		p
	BC (38)	BBD (75)	
	% of total fatty acids		
12:0 (lauric acid)	0.16 ± 0.09	0.22 ± 0.16	0.001*
14:0 (myristic acid)	1.69 ± 0.42	1.88 ± 0.46	0.036**
15:0 (pentadecylic acid)	0.19 ± 0.06	0.18 ± 0.05	0.134**
16:0 (palmitic acid)	21.16 ± 1.45	21.52 ± 1.66	0.270**
17:0 (margaric acid)	0.27 ± 0.11	0.25 ± 0.10	0.597*
18:0 (stearic acid)	4.63 ± 1.35	5.22 ± 1.34	0.031**
20:0 (arachidic acid)	0.15 ± 0.06	0.16 ± 0.06	0.219**
21:0 (heneicosanoic acid)	0.24 ± 0.14	0.22 ± 0.15	0.397**
22:0 (behenic acid)	0.26 ± 0.10	0.28 ± 0.11	0.587**
23:0 (docosanecarboxylate acid)	0.45 ± 0.20	0.35 ± 0.13	0.029**
Total SFAs	28.81 ± 3.13	30.02 ± 4.46	0.048*
14:1 (myristoleic acid)	0.13 ± 0.07	0.12 ± 0.06	0.456*
16:1 n-7 (palmitoleic acid)	3.07 ± 1.38	2.65 ± 1.22	0.022*
17:1 (heptadecenoic acid)	0.22 ± 0.05	0.20 ± 0.06	0.054**
18:1 n-9 (oleic acid)	41.05 ± 2.45	40.02 ± 2.96	0.068
20:1 n-9 (gondoic acid)	0.55 ± 0.13	0.55 ± 0.12	0.954
22:1 n-9 (erucic acid)	0.32 ± 0.16	0.22 ± 0.12	0.002**
Total MUFAs	45.53 ± 3.27	43.96 ± 3.96	0.039*
20:1 n-9 (gondoic acid)	0.55 ± 0.13	0.55 ± 0.12	0.954
18:2 n-6 *trans* (rumenic acid)	0.15 ± 0.15	0.18 ± 0.16	0.261*
18:2 n-6 (linoleic acid)	22.77 ± 2.71	23.31 ± 3.92	0.397**
18:3 n-6 (λ-linolenic acid)	0.23 ± 1.12	0.38 ± 1.17	0.714*
20:2 n-6 (eicosadienoic acid)	0.29 ± 0.09	0.30 ± 0.08	0.765
20:3 n-6 (dihomo-γ-linolenic acid)	0.04 ± 0.06	0.04 ± 0.01	0.483*
20:4 n-6 (arachidonic acid)	0.04 ± 0.01	0.03 ± 0.01	0.265*
Total n-6 PUFAs	23.98 ± 2.95	24.26 ± 5.32	0.747*
18:3 n-3 (α-linolenic acid)	0.96 ± 0.28	1.03 ± 0.30	0.315**
20:3 n-3 (dihomo- α-linolenic acid)	0.40 ± 0.21	0.29 ± 0.18	0.065**
20:5 n-3 (eicosapentaenoic acid)	0.20 ± 0.12	0.16 ± 0.10	0.255**
22:6 n-3 (docosahexaenoic acid)	0.07 ± 0.03	0.06 ± 0.03	0.372**
Total n-3 PUFAs	1.01 ± 0.77	1.05 ± 0.74	0.757*
Total PUFAs	25.11 ± 3.16	25.32 ± 5.06	0.865*
n-3/n-6 PUFA ratio	0.04 ± 0.02	0.04 ± 0.01	0.452**
20:4 n-6/20:3 n-6 ratio	0.88 ± 0.45	0.99 ± 0.09	0.551**
20:3 n-6/18:2 n-6 ratio	0.002 ± 0.00	0.002 ± 0.00	0.290*
18:1 n-9/ 18:0 ratio	8.921 ± 4.97	7.727 ± 2.69	0.015*

BC: breast cancer; BBD: benign breast disease; *Mann-Whitney Test; **T-Student Test

Total SFAs: 10:0.12:0. 14:0. 15:0. 16:0. 17:0. 18:0. 20:0. 21:0. 22:0. 23:0. 24.0

Total MUFAs: 14:1. 16:1. 17:1. 18:1. 20:1. 21:1. 24:1n-9

Total PUFAs: 18:2n-6. 18:3n-6. 20:3n-6. 20:4 n-6. 18:3n-3. 20:3n-3. 20:5n-3. 22:6n-3

Total n-6 PUFAs: 18:2n-6. 18:3n-6. 20:3n-6. 20:4 n-6

Total n-3 PUFAs: 18:3n-3. 20:3n-3. 20:5n-3. 22:6n-3

Table 4: Adjusted analysis of risk factors associated with breast cancer and benign breast disease.

	BC		BBD	
Variables	OR (95% CI)[a]	p*	OR (95% CI)[a]	p*
BMI (Kg/m^2)	2.75 (2.32 – 3.37)	0.888	2.21 (1.96- 2.53)	0.005
Total body fat (%)	2.69 (2.40 – 3.06)	0.888	3.07 (2.75 – 3.46)	0.024
Age at menarche (years)	2.34 (1.89 -3.11)	0.273	2.06 (1.77 – 2.50)	0.008
Age at first full pregnancy (years)	3.14 (2.84 -3.52)	0.004	3.26 (2.97 – 3.60)	<0.001
Age of first mammogram (years)	2.66 (2.54 -2.80)	0.467	2.57 (2.46 -2.69)	0.018
Physical activity	1.81 (1.32-3.49)	0.171	1.71 (1.35 – 2.61)	0.036

BC: breast cancer; BBD: benign breast disease; BMI: body mass index; CI: confidence interval; OR: odds ratio; * Derived from multiple logistic regression model adjusted for all the above variables.

3.3 Artigo 3 (Original): MTHFR and MTR Polymorphisms and Breast Cancer in Brazilian Women

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MTHFR and *MTR* Polymorphisms and Breast Cancer in Brazilian Women

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***Abstract*— Breast cancer (BC) is the second most common cancer, and mortality rates remain high among Brazilian women. However, the role of single nucleotide polymorphisms (SNPs) in one-carbon metabolism genes in breast cancer in Brazilian women is less clear. We aimed examine the association between the SNPs, in two genes in one-carbon metabolism alone and in cumulation, and the risk of breast cancer in an Brazilian population based case-control study of 257 breast cancer cases and 177 controls. Our hypothesis was woman who carries more risk genotypes has a higher susceptibility for developing breast cancer. Genotyping for MTHFR C677T and MTR A2756G polymorphisms were performed using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) method. Our results in population studied indicated that 677 C>T and 2756 A>G substitution does not appear to influence the risk of breast cancer. The cumulative effect was not observed with the OR being gradually elevated with increasing number of risk genotypes. However, larger studies are needed to further examine this interactions in this pathway and breast cancer risk in Brazilian women, as well in women of others nationalities.**

***Index Terms*— breast cancer, MTHFR C677T, MTR A2756G, one-carbon metabolism, polymorphisms.**

I. Introduction

Worldwide, breast cancer (BC) is the second most common cancer, the incidence rates vary widely across the world regions, nearly fourfold, and it is the most frequent cause of cancer death in women in less developed regions [1]. In Brazil, it is estimated 57,960 new cases for the year 2016 [2], and this disease is one of the main challenges faced by the Brazilian government, whose the mortality rate has progressively increased in recent years [3]

The disease is multifactorial, involving biological and endocrine factors, reproductive life, behavior and lifestyle [2], and the genetic risk factors can modify the risk of disease. Thus, alterations in the nucleotide sequences may be associated with cancer risk [4-6]. There are several single nucleotide polymorphisms (SNPs) in genes important in breast cancer, including the one-carbon metabolism pathway [7-10].

The methylenetetrahydrofolate reductase (MTHFR), one of the key enzymes in the one-carbon metabolism, catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate in 5-methyltetrahydrofolate [11, 12], while the enzyme methionine synthase (MTR), catalyzes the irreversible transfer of the methyl group of 5-methyltetrahydrofolate, promoting remethylation homocysteine to methionine [13].

The MTHFR 677 C>T and MTR *2756* A> G substitution have been considered to influence the enzymatic activity. Thence, studies have found association between the MTHFR C677T polymorphism [7, 14-20] and MTR A2756G polymorphism [7, 19, 21] in breast cancer, considered a genetic risk factor. Thus, reduced activity of these enzymes of one-carbon metabolism pathway could result in altered availability of methyl groups and impaired DNA methylation, and subsequently lead to cancer development [22].

The relationship between cumulative effect of genetic variants of MTHFR and MTR and breast cancer has not been extensively studied in the Brazilian population. Our hypothesis is that individual genetic variants when considered cumulatively can result in considerable effects. Thus, we aimed to examine the effect alone and in cumulation of MTHFR C677T and MTR A*2756*G polymorphism on the risk of breast cancer in Brazilian women.

II. Material and Methods

A. Study population and data collection

This hospital-based masked case-control study was developed at Odete Valadares Hospital in Belo Horizonte, Minas Gerais, Brazil, involving 257 breast cancer cases and 177 controls; more detail recruitment was described previously by our group [23, 24]. The research was approved by the National Committee of Ethics in Research, and all protocols used were approved and informed written consent for participation was gained from all patients.

B. DNA extraction

Genomic DNA was obtained from stored buffy coat. Briefly, buffy coats were digested using lysing solution,

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Madissen (0.1M Tris-HCl pH = 8.0; 0.4 M EDTA; 0.2% SDS; 1M NaCl; pH = 8.0), followed by addition of proteinase K (20 mg/mL) and incubated overnight at 37 °C. Then, the DNA was precipitated with saturated phenol and chloroform: isoamyl alcohol (24:1). Finally, cold isopropyl alcohol was added and homogenized slowly until the precipitation of DNA, which was dried at room temperature.

The extracted DNA was quantified in a spectrophotometer at 280nm and 260nm wavelength for evaluation of DNA purity and analyzed for integrity by comparing the bands obtained with the bands of different known concentrations of human DNA patterns by agarose gel electrophoresis 0.8%.

C. *Genotyping*

The polymorphisms of the genes under study were detected by the method restriction fragment length polymorphism (RFLP), after DNA amplification by polymerase chain reaction (PCR) (Table 1).

Table 1: Polymerase chain reaction and restriction protocols.

Gene	*Primer* (5'- 3')	PCR conditions	Restriction enzyme	Size of the fragment / Genotyping of patients
MTHFR C677T[a]	MTHFR677 f: TGA AGG AGA AGG TGT CTG CGG GA MTHFR677 r: AGG ACG GTG CGG TGA GAG TG	1 cycle 95°/5 min; 35 cycles to 55°/1 min, 72°/1 min, 95°/1 min; 1 cycle 72°/10 min.	HinfI	198 bp CC: 198 bp TT: 175 e 23 bp CT: 198, 175 and 23 bp
MTR A2756G[b]	MTR2756 f: CAT GGA AGA ATA TGA AGA TAT TAG AC MTR2756 r: GAA CTA GAA GAC AGA AAT TCT CTA	1 cycle 92°/2 min; 35 cycles to 56°/1 min, 72°/1 min 30 seg, 92°/1 min; 1 cycle 56° C/1 min; 72° C/1 min and 30 seg; 10° C/10 min.	HaeIII	189 bp AA: 189 bp GG: 159 and 30 bp AG: 189, 159 e 30 bp

Abbreviations: bp = base pair; min= minutes
[a] FROSST et al [25]
[b] LECLERC et al [26]

D. *Statistical Analysis*

Descriptive variables were compared between number of risk genotype using chi-square test for categorical variables and Kruskal-Wallis test for continuous variable. The association between MTHFR and MTR genotype, and breast cancer were assessed by logistical regression model with results expressed as Odds Ratios (ORs) and their 95% confidence intervals (CIs) after adjusting for age and menopausal status. Hardy–Weinberg Equilibrium was tested to compare the observed with expected genotype frequencies. Analyses were performed by using SPSS® software, version 20 (SPSS INC. Chicago, IL. USA). All P-values were two sided, and a P-value <0.05 was considered statistically significant.

III. Results

Characteristics of the study population are shown in Table 2. Overall, women did not differ in function of the number of risk genotypes, suggesting homogeneity of the sample. A total of 257 breast cancer cases and 177 controls were included in final analyses.

The results of the selected SNPs in one-carbon metabolism genes and the breast cancer risk were shown in table 3. The MTH A2756G was associated with breast cancer, with decreased risk, in the crude analyses (OR= 0.551, 95% CI: 0.335 – 0.908). After adjustment for age and menopausal status the significance statistical was lost (OR= 0.602, 95% CI: 0.335 – 1.028). We did not observe any difference between increase of the number of risk genotypes and breast cancer risk, indicating that the SNPs of one-carbon metabolism genes evaluated not associated with this disease in Brazilian women included in this study.

Table 2: Characteristics of participants included in the study.

Variables	Number of risk genotypes			
	0/2 (n=163)	1/2 (n=184)	2/2 (n=39)	p-value
Age (mean ± SD)	51.44 ± 11.18	52.25 ± 12.07	54.9 ± 10.98	0.151[a]
Age of menarche				
≥ 13	99	108	27	0.514*
< 13	62	74	12	
Menopausal Status				
premenopausal	68	69	11	0.277*
postmenopausal	94	112	28	
Family history of BC				
No	134	146	32	0.988*
Yes	29	33	7	

[a]The Kruskal-Wallis test;*The chi-square test.

Table 3: Associations between MTHFR and MTR single nucleotide polymorphisms (SNPs) and breast cancer in Brazilian women.

	Case [n (%)]	Control [n (%)]	*OR* (CI 95%)[a]	*OR* (CI 95%)[b]
*MTHFRC677T**				
CC	145 (56.6 %)	98 (55.4 %)	1	1
CT+TT	111 (43.4 %)	79 (44.6 %)	1.053 (0.716 – 1.549)	0.979 (0.956 – 1.003)
*MTH A2756G***				
AA	165 (71.4 %)	127 (81.9 %)	1	1
AG+GG	66 (28,6 %)	28 (18.1)	**0.551 (0.335 – 0.908)**	0.602 (0.335 – 1.028)

Results by logistic regression [a]Crude, [b]Adjusted for age and menopausal status. *MTHFRC677T information was available: 256 (99.61%) breast cancer cases and 177 (100%) controls. **MTH A2756G information was available: 231 (89.88%) breast cancer cases and 155 (87.57%) controls.

Table 4: Cumulative effect of SNPs susceptibility for breast cancer.

Number of risk genotype[a]	Case [n (%)]	Control [n (%)]	*OR* (IC 95%)*	p-value
0/2	88 (38,1)	75 (48,4)	1	
1/2	119 (51,5)	65 (41,9)	1.116 (0.519 – 2.399)	0.780
2/2	24 (10,4)	15 (9,7)	0.694 (0.323 – 1.493)	0.351

[a]Based on the two SNPs. *Data were calculated by logistic regression adjusted for age and menopausal status.

IV. Discussion

The results from this study demonstrated that family history does not appear to have an association with increasing the number of risk genotypes in SNPs of one-carbon

metabolism. These data suggest that women included in the study did not differ in general characteristics. Recent study concluded that clinical management should not differ between women with and without family history, because the authors not find evidence to support an association between family history of breast cancer and severity and breast cancer-specific mortality [27].

Results of studies with women from different populations are still inconclusive. Some found an association between MTHFR C677T variant alleles and increased risk for breast cancer [14-16, 18]; while others detected a reduced risk [28]; while others found no significant relationship [29].

Polymorphism studies in MTR A2756G in relation to risk of breast cancer have reported mixed results, including no association [30-33], inverse relationship [10, 34] or positive association [19, 21] with the variant allele.

Hence, each of these variants was found to be independently and moderately associated with breast cancer risk in different populations, thus, the combinations of risk alleles could be a cumulative effect on breast cancer, and we tested this hypothesis in Brazilian women.

In this study, no significant associations were detected between breast cancer risk and SNPs evaluated, as well the cumulative effect of these SNPs by counting the number of genotypes associated with breast cancer risk. In breast cancer [35] and other types of cancer, patients carrying higher number of risk genotypes shown increased risk for colorectal cancer [36], gastric carcinoma [37], and thyroid cancer [38].

Recently, models have been developed to predict the risk of breast cancer in women. These models may consider the SNP-SNP interactions in breast cancer [39] as well as genetic variants and established risk factors [40].

One point worth mentioning is the effect of ethnic in the associations of these SNPs described in the literature, the data vary greatly depending on the population, in which the study is conducted, suggesting that these SNPs may have different effects in different populations. This fact can justify incidence rates varying widely across the world regions. Similar studies can be conducted in different ethnic groups and populations to provide more insights into the molecular pathophysiology of breast cancer.

V. Conclusion

Taken together, in population studied, the MTHFR 677 C>T and MTR 2756 A> G substitution does not appear to influence the risk of breast cancer, in Brazilian women.

Similar studies can be conducted in different ethnic groups and populations to provide more insights into the molecular pathophysiology of breast cancer.

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3.4 Artigo 4 (Original): Benign Breast Disease and Associated Factors in Women Attending in a Public Hospital.

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Benign Breast Disease and Associated Factors in Women Attending in A Public Hospital

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Abstract **- The aim of this study was to present the main features of women with benign breast disease (BBD) and the factors associated with these diseases. Our study was a case-control, masked and hospital-based study. Sociodemographic, clinical and gynecological, as well as obstetrical, anthropometric and lifestyle data were collected from the BBD-diagnosed women and controls. Most participants were diagnosed with fibroadenoma. The three protective factors against the development of BBD included parity, late menarche and breastfeeding. In general, these major protective effects are connected to the endogenous hormone levels and main reproductive events, which are more difficult factors to control. reproductive events, which are more difficult factors to control. The type of benign breast disease, age of diagnosis, degree of education and woman's age seem to contribute to this relationship.**

Index Terms **- benign breast disease, breast cancer, risk factors, Brazilian women**

I. INTRODUCTION

Benign breast disease (BBD) includes the heterogeneous group of lesions with a variety of histological subtypes and occurs more frequently than breast cancer (BC). The etiology has been attributed to hormone level changes during the life of women and to the reproductive cycles, in particular, which contributes to the differentiation in breast structure and cellularity [1, 2]. Therefore, recognizing benign breast lesions becomes crucial to facilitate selecting the most suitable treatment plan for each type [3].

At present, the main challenge it to gather data on the epidemiology of BBD, as a standardized histologic classification system commonly available to the scientific community is absent, and realistic estimates of the prevalence of this condition in the general population are lacking [4].

Cohort studies, however, demonstrated a higher risk of breast cancer development in women who had benign breast disease [5, 6]. From a recent meta-analysis multiethnic, BBD appeared to be the common factor that raised the risk of BC by approximately 1.17 for non-proliferative disease, 1.76 for disease unspecified and 3.93 for unspecified atypical proliferative disease without atypia, 2.07 for benign breast hyperplasia [4]. Further, in African women with proliferative disease atypia a three-fold increased risk of subsequent development of breast cancer was observed compared with those who had non-proliferative disease [7].

Pankratz and co-workers, therefore, developed a model which could predict the likelihood of BC developing in women, right at the time of benign biopsy. Based on the women, right at the time of benign biopsy. Based on the recognized histological features, as well as the patients' demographic and clinical characteristics, this model could well be a significant step in individualized risk prediction of the BC in women BBD [8].

Some studies suggested that on average, the time from the initial diagnosis of BBD to breast cancer diagnosis was between 6.4 and 10.7 years [4, 5, 7]. As the risk of recurrence lies in the women population with BBD, special attention must be paid to the reaction of these women to the diagnosis, because the treatment directly influences their quality of life [9].

Clinical evaluation, therefore, becomes critical to the exclusion of malignancy, and the clinical management must necessarily be differentiated, based mostly on patient age. Young women (< 35 years) with BBD, do not always opt for nodule excision. The size, location and the patient desire to undergo the procedure must be considered. In consenting patients, fine-needle aspiration has been found to be the most suitable procedure. At present, for patients over 35 years, the treatment is based on the triple diagnosis, as clinical, imaging and histological, with surgical indication. This is justified as the incidence of BC tends to progressively increase with age [3].

Breast cancer has been recognized as a public health problem, because of its repercussions on the social, health / morbidity, psychological and economic factors in society [10]. In the breast disease process, the psychosocial impact is evident by high anxiety levels and or depression and patient concern regarding body image and sexuality [9]. Another major concern is the high cost, whether direct (outpatient, hospital, drugs) or indirect (absenteeism, early retirement, loss of productivity) [10]. Thus, there is an urgent need for early detection and diagnosis, followed by correct treatment of patients at risk, in order to reduce the complications and increase survival.

This article describes the main characteristics of women with BBD, given assistance in a public hospital. Besides, the

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BBD-related factors have been identified compared with the control group.

II. MATERIAL AND METHODS

This is a case-control, masked and hospital-based study, conducted on the women attending in the Mastology or Gynecology Service of Motherhood Odete Valadares, Hospital Foundation of Minas Gerais (FHEMIG) of Belo Horizonte, Minas Gerais, Brazil, in 2006 [11, 12].

Women undergoing routine tests, breast or gynecologic surgery at the hospital were invited to participate in the study by signing the Term of Free and Clear consent form. The study was approved by the National Committee for Research Ethics, in the advice number 1889/2005, and was conducted according to Declaration of Helsinki.

All patients with a confirmed diagnosis of benign breast disease by pathological examination were included in this study; those excluded were patients below 20 years, those diagnosed prior with breast cancer or benign breast disease and those lacking complete data, like patients with incomplete questionnaires, absence of the pathological examination results, and lack of recent mammography (maximum of two years prior to the interview date).

The volunteers were categorized into two groups: group benign breast disease (GBBD), composed of women with a histopathologic diagnosis of non-proliferative breast tissue changes or other benign proliferative breast diseases and the group control (GC) which included women who had undergone a recent mammogram with Class I or II disease according to the BI-RADS classification criteria of the Brazilian Society of Mastology. The final sample thus involved 338 women.

We utilized a previously validated questionnaire for the population study [13]. Patient identification, including data sociodemographic, clinical and gynecological data and anthropometric and lifestyle characteristics was collected.

Weight was measured on an electronic scale Tanita® - Tanita Body Fat Monitor Scale (Model TBF 531®, Tanina Corporation of America, Illinois, USA), with maximum capacity of 150 kg and sensitivity of 100 g. The height was recorded with a vertical anthropometer (Alturaexata®), having a rigid rod with a scale bilaterally showing 35 to 213 cm in 1 mm divisions. These measurements were performed according to the standard recommendations by Jelliffe [14] and Frisancho [15]. Body mass index (BMI) was then calculated using the formula: weight (kg) / height (m^2) and overweight was defined as BMI > 25.0 kg / m^2 [16].

The following cutoffs were considered risk factors: age of menarche less than 12 years; first pregnancy after 30 years; first mammogram indicated before of 40 years [10].

The description of the population was initially done based on the type of benign breast disease and the age of diagnosis. The descriptive analysis of general characteristics was presented based on the relative frequency distributions. Evaluation of the factors related to the BBD was performed using binary logistic regression. The results are shown as *odds ratios* (OR) and confidence intervals of 95% (CI 95%) for crude and adjusted models by age and education. The significance level was set at 5%. The data were analyzed using SPSS for Windows (SPSS INC. Chicago, IL. USA), version 20.0.

III. RESULTS

Depending on the difficulty of the standardized histological classification for BBD, a survey of the clinical and histological characteristics was proposed, as seen in Table 1. We reviewed the literature according to the findings found in this study from the analysis of the pathologist from our staff. We have also prepared illustrative images of different types of benign breast lesions of the women in this study (Figure 1 and 2).

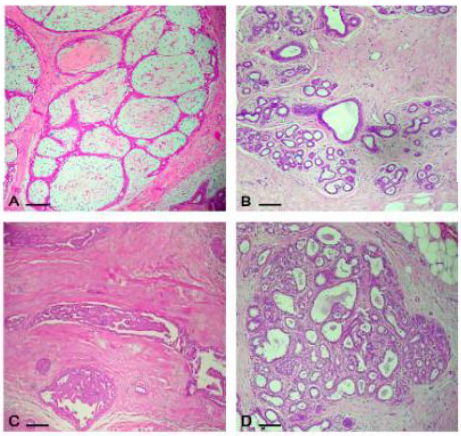

Fig 1 Illustrative images of different types of benign breast lesions in women. A: Fibroadenomas; B: Fibrocystic changes; C: Ductal hyperplasia; D: Adenosis. Scale bars: 50 µm

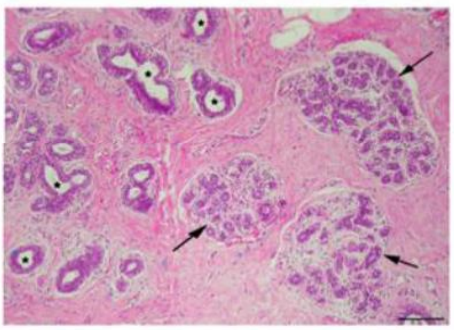

Fig 2 Photomicrograph showing the mammary lobules transition region of normal tissue for the hyperplastic. On the left side the usual ductal hyperplasia (*) on the right side and normal tissue. The arrows indicate the normal tissue. Scale bars: 50 µm

The study included 338 women undergoing breast cancer treatment at a referral hospital. Among these, 159 (47.1%) had benign breast disease (GBBD) and 179 (52.9%) were controls (GC). Most of the women with BBD were diagnosed with fibroadenoma (43.9%). Regarding the age of diagnosis, younger women (39.4 ± 12.9 years) showed fibroadenoma, while the older ones (52.7 ± 14.8 years) had atypical hyperplasia (Table 2).

Table 1 Main clinical and histopathological features of benign breast lesions [17], [18]

Pathological diagnosis:	Characteristics: Clinics	Histopathological
Fibroadenomas	- Small nodule (up to 3 cm), single; - Hard consistency and elastic; - Mobile on palpation; - Painless; - Slow growth;	- Lobed surface, well delimited; - Hyperplasia of lobules normal;
Fibrocystic breast changes	- Variable size palpable mass; - Margins generally ill-defined;	Alone or combined presence: - stromal fibrosis; - apocrine metaplasia; - adenosis;
Proliferative disease without atypia	- Irregular growth; - No evident cell borders;	- Cells proliferate in addition 3 to 4 layers above the basement membrane, tending to distend and fill the ducts involved; - Cell orientation can be in snails, arches and bridges; - Ductal hyperplasia of usual type;
Benign phyllodes tumor	- Unilateral mass (low 4 cm); - Mobile on palpation; - Painless;	- Margins are not so defined, with lobulated border; - Standard "cloverleaf"; - Mesenchymal component is hypercellular, mainly around the glandular structures; - Minimal or absent cell atypia;
Atypical ductal hyperplasia	- Small lesions (menor 2mm); - Hard consistency; - Nonpalpable; - Asymptomatic;	- Intraductal proliferation of monomorphic cells, with regular distribution; - Homogeneous cell proliferation; - Formation of micropapilas; - Hyperchromasia may be present; - Hiperplasia ductal atipica;
Atypical lobular hyperplasia	- Nonpalpable; - Asymptomatic;	- Multifocal and bilateral distribution; - The cells may extend through the ducts.

Table 2 Percentage of different histological subtypes of benign breast disease according to the mean age of diagnosis (n=157)

Type of benign breast disease:	n (%)	Age at diagnosis Dean (SD)
Fibrocystic breast changes	19 (12.1)	47.7 ± 11.9
Fibroadenomas	69 (43.9)	39.4 ± 12.9
Other breast lesions nonproliferative	37 (23.5)	46.7 ± 12.0
Proliferative lesions without atypia	24 (15.2)	49.50 ± 11.2
Benign phyllodes tumor	4 (2.5)	44.5 ± 6.4
Atypical hyperplasia	4 (2.5)	52.7 ± 14.8

Women between 40 and 59 years old and with low degree of education (< 5 years) were more likely to have BBD. However, no relationship of the disease with marital status, occupation, smoking, alcohol consumption or physical activity was noted (Table 3).

In the sample were observed crude associations between breastfeeding, nulliparity, age at menarche, overweight, the first mammography age and BBD. After adjustment for age and education, only age at first mammogram did not remain associated (Table 4).

Table 3 Factors sociodemographic, behavioral and benign breast disease

Variables	Groups GBBD (n=159) [n (%)]	GC (n=179) [n (%)]	OR (95% CI)
Age (years)			
20 - 39	48 (30.1)	29 (16.2)	1
40 - 59	94 (59.2)	126 (70.4)	2.337 (1.078 – 5.065)
≥ 60	17 (10.7)	24 (13.4)	1.053 (0.536 – 2.071)
Marital status			
Married	87 (54.7)	91 (51.2)	1
Separate / Divorced	16 (10.0)	33 (18.5)	1.593 (0.735 – 3.454)
Single	44 (27.7)	34 (19.1)	0.808 (0.318 – 2.052)
Widow	12 (7.6)	20 (11.2)	2.157 (0.927 – 5.017)
Education (years)			
< 5	26 (16.3)	47 (26,2)	1
≥ 5	133 (83.7)	132 (73,8)	0.549 (0.321 – 0.939)
Occupation			
Retired	11 (6.9)	14 (7.8)	1
Education service	8 (5.1)	1 (0.6)	0.127 (0.016 – 1.548)
Health Service	8 (5.1)	7 (3.9)	0.229 (0.021 – 2.456)
Social service	51 (32.0)	52 (29.2)	0.196 (0.022 – 1.738)
Domestic Service	81 (50.9)	104 (58.5)	0.148 (0.017 – 1.289)
Smoking			
Current	21 (19.9)	28 (16.0)	0.615 (0.201 – 1.887)
Former	6 (5.6)	13 (7.4)	0.792 (0.422 – 1.488)
Never	79 (74.5)	133 (76.6)	1
Alcohol Consumption			
No	87 (82.8)	151 (85.8)	1
Yes	18 (17.2)	25 (14.2)	1.250 (0.645 – 2.420)
Physical activity			
No	134 (84.3)	154 (86.5)	1
Yes	25 (15.7)	24 (13.5)	0.835 (0.455 – 1,531)

GBBD: group benign breast disease; GC: group control; BMI: body mass index; OR: odds ratio; CI: confidence interval.

IV. DISCUSSION

Most of the biopsies showed non-proliferative lesions (79.8%), including fibrocystic changes of the breast, fibroadenoma, and other non-proliferative breast lesions. About 17.7% of the biopsies revealed proliferative lesions without atypia (proliferative lesions without atypia, benign phyllodes tumor) and 2.5% had atypical hyperplasia (Table 2). Our results are concurrent with those of other studies which also found the same profile of benign breast lesions, showing greater occurrence of non-proliferative lesions and lower frequency of proliferative with atypia [5-7].

Noteworthy is also the breast lesions that occur in most cases are benign. Thus, fibroadenoma, considered a non-proliferative lesion, is the commonest among the benign tumors in female breast, and the most frequent in women of reproductive age, particularly in the thirddecade of life [17]. Indeed, our results corroborate with the description given above, in that fibroadenoma was diagnosed early in women, in their third decade of life.

In this study, the mean age of the women diagnosed with BBD at the time of the first breast biopsy was 42.2 ± 12.8 years. Other studies reported the average age being slightly higher, at 48.6 years for African women [7], and 46.6 years for women included in a multiethnic meta-analysis [4].

A few studies also indicated that women having a previous history of benign breast disease had a higher risk of developing breast cancer, in particular those with proliferative breast disease, over those with nonproliferative type [4, 7]. Hartmann and co-workers highlighted the risk factors for breast cancer after the diagnosis of benign breast disease, which include the histologic classification of a benign breast tissue lesion and having a family history of breast cancer [5].

Table 4 Characteristics clinical, gynecological, obstetric and anthropometric with benign breast disease

	Total			
	GBBD	GC	OR (95% CI) Crude	OR (95% CI) Ajusted
Nulliparity				
Yes	40	17	1	1
No	117	160	0.311 (0.168-0.575)	0.397 (0.205-0.767)
Menarche				
< 12 years	42	27	1	1
≥ 12 years	122	149	0.483 (0.281-0.831)	0.542 (0.311-0.946)
Menopause				
No	100	104	1	1
Yes	57	73	0.812 (0.522-1.263)	1.264 (0.736-2.170)
Age of first full pregnancy				
< 30 years	147	101	1	1
≥ 30 years	13	17	1.909 (0.888-4.106)	1.957 (0.900-4.256)
Age of first MMG				
≤ 40 years	106	100	1	1
> 40 years	51	77	0.625 (0.400-0.977)	0.789 (0.471-1.324)
Breastfeeding				
No	58	27	1	1
Yes	99	149	0.305 (0.181-0.514)	0.361 (0.208-0.628)
Stillbirth				
No	140	154	1	1
Yes	12	23	0.574 (0.275-1.196)	0.671 (0.317-1.418)
Abortion				
No	100	115	1	1
Yes	53	62	0.983 (0.624-1.548)	1.199 (0.742-1.937)
Use of oral contraceptives				
No	52	61	1	1
Yes	105	114	1.080 (0.685-1.703)	1.048 (0.655-1.675)
Hormone replacement therapy				
No	136	145	1	1
Yes	19	29	0.699 (0.374-1.304)	0.825 (0.436-1.563)
Overweight (BMI)				
No	83	62	1	1
Yes	74	117	0.480 (0.309-0.746)	0.540 (0.343-0.849)

GBBD: group benign breast disease; GC: group control; MMG: mammography; OR: odds ratio;
CI: confidence interval. Adjusted for age and education.

It is now concluded that the precursors involved in breast cancer development may already exist in benign breast disease. This finding arises from the observation that breast cancer occurred in the same breast, particularly in those women with earlier diagnosis of proliferative disease with atypia [5].

In this study, after adjusting for covariates, the birth of at least one child acted as a protective factor against the development of BBD, as were late menarche and breastfeeding. Women with benign breast disease were then tested to identify the reproductive factors, hormonal and lifestyle aspects connected with the risk of breast cancer development in the future. In light of this finding, patients with the first live birth before 25 years of age and three or more pregnancies had an OR value of 0.49 for breast cancer, implying a protective effect of early age at first pregnancy and higher parity [19].

The literature reports age of menarche lower than 12 years as a risk factor for breast cancer, probably due to the prolonged exposure of the breast epithelium to estrogen and progesterone induced by the early onset of the regular menstrual cycles and ovulation. Our results emphasize that delayed menarche decreases the length of exposure time to the endogenous sex hormones, thereby protecting the women of this study, against the development of BBD, because the age of menarche is a chronological index of the initiation of ovarian activity [20].

Estrogen and progesterone ovarian govern the processes of both epithelial cell proliferation in the breast tissue, as well as the rise of hormones like prolactin and IGF-1. During adolescence and adulthood, these may be the major factors that determine the risk of developing breast cancer by increasing those cells which trigger the promotion of carcinogenesis and tumor growth [21].

Two likely mechanisms have been suggested to show that mammary carcinogenesis is linked with estrogen production. The first is dependent on the estrogen receptor, which mediates the stimulation of the cell proliferation in the breast, increasing the mutation rate. Finally, the mechanism, independent of the estrogen receptor-mediated genotoxic metabolites of estrogen, results in more DNA mutations. If either of these pathways or both are active, the mutations will accumulate over time, and thus induce neoplastic transformation [22].

Further, breastfeeding is definitely a protective mechanism, because it is during this time that the amenorrhea induced by the lactating mammary cells are exposed to the effect of the sex hormones for a lesser time span, as they decrease at this period. Another mechanism proposed is that the cells which has suffered DNA damage got eliminated, via strong exfoliation through the breast tissue and epithelial cell apoptosis [23].

Pregnancy also offers protection which is mediated by breast tissue differentiation. The pregnancy-related epigenetic changes in mammalian cells lower their susceptibility to tumor formation, because gestation triggers a local reprogramming and some possibly unsuitable genes are muted, like the ones connected with cell proliferation [24].

Thus, age at menarche, age of first pregnancy, and breastfeeding appear to have no bearing on fibrocystic breast disease or fibroadenoma [25]. In contrast, another study reported that breast tissue density alone was associated with benign proliferative breast disease, showing around twice the risk (OR = 1.91). However, all other possible epidemiological risk factors that were evaluated are not associated, including lifestyle, socioeconomic and anthropometric factors, as well as the reproductive and menstrual history [26].

In this study, the overweight was significantly associated with a lower risk of developing BBD in both models, crude and adjusted for age and education. At present, there is a gap in knowledge regarding the BMI and the BBD. However, recent research showed an inverse relationship between BMI and the benign pathologies evaluated, i.e. the patients with increasing BMI had a lower incidence of BBD [27].

V. CONCLUSIONS

It is a well accepted fact that the BBD is very common, although the incidence is not well documented in the literature, probably because its importance is underestimate. In light of the results of this study, the mostly protective factors linked with the BBD are related to the concentrations of the endogenous hormone and main reproductive events like menarche and parity, which are the more difficult factors to control. However, breastfeeding should be encouraged, as it contributes to a lowering of the incidence of BBD and possibly in the subsequent development of breast cancer in the future.

Therefore, further studies in this field are warranted to raise our awareness of the behavior of benign breast disease in this population. This will enable the identification of the high-risk women who could benefit from heightened vigilance and, ultimately, clear diagnosis and early treatment.

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4. CONCLUSÕES GERAIS

As mulheres com câncer de mama e com doença benigna da mama agrupadas neste estudo, apresentaram concentração de ácidos graxos do tecido mamário, bem como as características sociodemográficas, antropométricas, reprodutivas, ginecológicas e de estilo de vida diferenciadas quando comparadas ao grupo controle. Os outros parâmetros avaliados não diferiram estatisticamente entre os grupos avaliados.

Considerando o consumo alimentar, o presente estudo não encontrou diferenças significativas entre as mulheres avaliadas. Ainda assim, é importante destacar que a maioria dos estudos que avaliam a ingestão alimentar e o risco de desenvolvimento do câncer de mama são também conduzidos com mulheres durante a meia-idade ou idade mais tardia. Porém, devemos lembrar que as mulheres nesta fase da vida, podem se encontrar em um período em que o tecido mamário não é mais vulnerável a influências dos carcinógenos.

Sugere-se que estudos de coorte sejam conduzidos visando acompanhar mulheres durante um longo período de tempo, buscando compreender os possíveis alimentos protetores e o risco futuro de desenvolvimento de câncer de mama. Tal abordagem se justifica também em função da dificuldade de se estimar de maneira precisa o tempo de exposição aos fatores de risco associados ao câncer de mama, principalmente em estudos nas quais as coletas de dados são pontuais apenas, em um único momento.

Outro aspecto importante do presente trabalho foi a não associação significativa entre os polimorfismos avaliados e os grupos estudados. Especificamente, quando investigamos as combinações de alelos de risco que poderiam gerar um efeito cumulativo no câncer de mama, ainda assim, não encontramos associações significativas. Estudos que avaliam a presença de SNP e o risco de doenças crônicas não transmissíveis na população brasileira ainda são escassos e somado a este fato, temos uma população com diferentes etnias e oriundas

de diversas regiões geográficas, sendo fatores estes que podem estar envolvidos na variabilidade dos resultados encontrados na literatura, até o momento.

Estes fatores precisam ser melhor explorados em estudos futuros, uma vez que, a etnia é um fator importante, e assim, estudos em diferentes grupos étnicos e populações são necessários para fornecer mais informações sobre a fisiopatologia molecular do câncer de mama.

Anexo 1: Aprovação do Comitê de Ética

FHEMIG
FUNDAÇÃO HOSPITALAR DO ESTADO DE MINAS GERAIS

DECLARAÇÃO

Declaro que o projeto de pesquisa intitulado **"Correlação entre fatores dietéticos, clínicos e genéticos e a ocorrência de câncer de mama em mulheres atendidas pelo Serviço de Mastologia da Maternidade Odete Valadares em Belo Horizonte, MG"**, sob responsabilidade da pesquisadora professora Renata Nascimento de Freitas, da Universidade Federal de Ouro Preto, foi **analisado e aprovado** pelo Comitê de Ética em Pesquisa da Fundação Hospitalar do Estado de Minas Gerais (CEP/FHEMIG), no dia 14 de julho de 2005, conforme parecer nº 310, e pelo Comitê Nacional de Ética em Pesquisa do Conselho Nacional de Saúde do Ministério da Saúde (CONEP/CNS/MS), no dia 29 de novembro de 2005, conforme parecer nº 1889/2005.

Belo Horizonte, 02 de fevereiro de 2007.

Dr. Robespierre Queiroz da Costa Ribeiro
Coordenador do CEP-FHEMIG

Anexo 2: Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DE OURO PRETO
MATERNIDADE ODETE VALADARES

1- DADOS DE IDENTIFICAÇÃO DO SUJEITO DA PESQUISA OU RESPONSÁVEL LEGAL:

NOME DO PACIENTE: _____

DOCUMENTO DE IDENTIDADE Nº: _____ÓRGÃO EXPEDIDOR:_____

SEXO:_____ DATA NASCIMENTO:____/____/_____

ENDEREÇO: _____

BAIRRO: _____ CIDADE: _____

TELEFONE: (__) _____

RESPONSÁVEL LEGAL: _____

NATUREZA (grau de parentesco, tutor, curador etc.): _____

DOCUMENTO DE IDENTIDADE Nº: _____ÓRGÃO EXPEDIDOR:_____

SEXO:_____ DATA NASCIMENTO:____/____/_____

7.1 2- DADOS SOBRE A PESQUISA CIENTÍFICA:

TÍTULO DO PROJETO: **CORRELAÇÃO ENTRE FATORES DIETÉTICOS, CLÍNICOS E GENÉTICOS E A OCORRÊNCIA DE CÂNCER DE MAMA EM MULHERES ATENDIDAS EM SERVIÇO PÚBLICO DE MAMOGRAFIA EM BELO HORIZONTE, MG.**

COORDENADORA: PROFESSORA RENATA NASCIMENTO DE FREITAS / ESCOLA DE NUTRIÇÃO / UFOP
AVALIAÇÃO DO RISCO DA PESQUISA: RISCO MÍNIMO
DURAÇÃO DA PESQUISA: 18 MESES

3- REGISTRO DAS EXPLICAÇÕES DO PESQUISADOR AO PACIENTE OU SEU REPRESENTANTE LEGAL SOBRE A PESQUISA:

A pesquisa que a senhora está sendo convidada a participar tem como objetivos investigar os fatores da dieta, da composição corporal e genéticos que

fazem com que uma mulher tenha mais ou menos chances de desenvolver câncer de mama.

Nesta pesquisa cada participante deverá responder a um questionário, que será aplicado pela equipe no dia da consulta no hospital. De cada participante serão também tomadas medidas de peso, altura, circunferência da cintura, circunferência do quadril e das dobras de gordura subcutânea. Se por acaso, a senhora for submetida à cirurgia ou a biópsia para diagnóstico ou tratamento, serão coletadas uma amostra de sangue (10 mL) e uma amostra de gordura da mama (0,5 a 1,0 g) retirada normalmente nestes procedimentos. O sangue e a amostra de gordura serão enviados para o Laboratório de Epidemiologia Molecular da Escola de Nutrição da Universidade Federal de Ouro Preto para extração de DNA (material genético) e análises bioquímicas. No DNA serão pesquisados os genes da *MTHFR, TYMS, MTR, APOE, LDL-R, PPAR* e *FAS*. Estes genes estão relacionados ao metabolismo de gorduras e de ácido fólico (uma vitamina) que são consumidos na dieta. Existem evidências de que alguns tipos de gorduras e o ácido fólico da dieta possam estar associados com o risco de câncer de mama e é isto que queremos pesquisar. Na gordura da mama serão pesquisados os tipos de ácidos graxos (constituintes das gorduras) presentes. A partir da análise dos resultados destes dados é que pesquisaremos que características podem influenciar no desenvolvimento do câncer de mama. O material coletado para este estudo receberá um código e apenas a professora Renata Nascimento de Freitas da Universidade Federal de Ouro Preto saberá a origem do mesmo.

Este material será utilizado apenas para os estudos descritos acima e ao final, será descartado. Em nenhum momento desse estudo, as pessoas que estarão trabalhando com este material saberão que é seu, garantindo o sigilo de seus dados. Nenhuma outra pessoa ou instituição, que não aquelas envolvidas no presente projeto, terá acesso aos questionários ou dados individuais gerados por esta pesquisa. Os resultados deste trabalho serão publicados apenas em veículos de divulgação científica (revistas especializadas e congressos) garantindo-se o anonimato dos participantes. Sua participação ou não neste estudo não influenciará de nenhuma forma no tipo e na qualidade do atendimento médico que você está recebendo ou poderá receber no futuro. Você poderá solicitar aos pesquisadores, a qualquer momento, o seu desligamento do estudo e a retirada dos seus dados.

Você poderá ter conhecimento, se quiser e no momento que desejar, dos resultados da avaliação nutricional e das análises bioquímicas e genéticas. Se necessário e se for de seu interesse, nossa equipe agendará uma consulta para que a senhora receba aconselhamento genético e aconselhamento nutricional.

É através deste tipo de pesquisa e da divulgação dos resultados, que esperamos poder aumentar nosso conhecimento sobre os fatores que aumentam ou diminuem os riscos de desenvolvimento de câncer de mama. Sua participação poderá ajudar a melhorar os conhecimentos necessários para melhor orientar programas de prevenção que poderão contribuir para diminuir a ocorrência deste câncer que é o que mais mata mulheres em todo mundo.

Caso a senhora queira se informar de mais detalhes sobre a pesquisa agora, ou no futuro, poderá entrar em contato com a Profa. Renata N. Freitas na Escola de Nutrição da UFOP pelo telefone (31) 3559 1838 ou por ligação gratuita para o telefone 9 031 31 3552 0121 por e-mail: rfreitas@enut.ufop.br **. Obrigada!**

4- ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE GARANTIAS DO SUJEITO DA PESQUISA:

Acesso, a qualquer tempo, às informações sobre procedimentos, riscos e benefícios relacionados à pesquisa, inclusive para esclarecer eventuais dúvidas.

Liberdade de retirar seu consentimento a qualquer momento e de deixar de participar do estudo, sem que isto traga prejuízo à continuidade da assistência.

Acesso a qualquer tempo aos resultados desta pesquisa com aconselhamento genético e/ou nutricional se necessário.

Salvaguarda da confidencialidade, sigilo e privacidade.

5- CONSENTIMENTO PÓS – ESCLARECIMENTO

Declaro que, após convenientemente esclarecida pelo pesquisador e ter entendido o que me foi explicado, consinto em participar do protocolo da pesquisa acima especificado.

Belo Horizonte, de de 200 .

Assinatura do sujeito da pesquisa ou representante legal.

Assinatura do pesquisador (carimbo ou nome legível)

Anexo 3: Questionário de avaliação dos fatores de risco para câncer de mama

Questionário de Avaliação dos Fatores de Risco para Câncer de Mama

Data da avaliação:_____ Número:_____
Prontuário:_____

A.IDENTIFICAÇÃO

1.Nome:_____
Identidade:_____ Órgão expedidor:_____
2.Idade:_____ Data Nascimento:_____
3.Residência atual:Rua:_____N°_____
Bairro:_____ Cidade:_____
Tempo de residência:_____ anos Zona: (U) Urbana (R) Rural
4.Residência anterior:Rua:_____N°_____
Zona: (U) Urbana (R) Rural Bairro:_____Cidade:_____
5.Cidade do nascimento:_____Estado:_____
Zona: (U) Urbana (R) Rural Tempo de residência:_____ anos
6. Situação conjugal:(IBGE/2000)

(1) Casada/ Consensual (2) Separada/Divorciada/Desquitada
(3) Solteira (4) Viúva

7.Escolaridade: (IBGE/2000-INCA/2000)

(1) Não alfabetizada
(2) Alfabetizada/Alfabetização de adultos
(3) Antigo primário incompleto/1 -3 série
(4) Antigo primário incompleto/Elementar completo/1-4-série
(5) Ginásio incompleto/5-7 série
(6) Ginásio completo/5-8 série
(7) Antigo clássico incompleto/Normal incompleto/Ensino médio incompleto
(8) Antigo clássico completo/Normal completo/Ensino médio completo
(9) Superior/Superior mestrado/Superior doutorado

8.Ocupação:_____
9.Renda líquida mensal:_____ 10.N° de membros da família:_____

B.HISTÓRIA GINECO-OBSTÉTRICA

1.Idade da menarca:_____
2.Idade da primeira gestação completa:_____
3. Número de gestações com filhos vivos:_____
4.Número de abortos:_____
5.Número de natimortos:_____
6.Já amamentou? ( ) SIM ( ) NÃO

Quanto tempo amamentou seus filhos? (OMS/1992)

Filho 1	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses	**Filho 2**	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses
Filho 3	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses	**Filho 4**	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses
Filho 5	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses	**Filho 6**	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses
Filho 7	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses	**Filho 8**	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses
Filho 9	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses	**Filho 10**	( ) AME_____ meses ( ) AMP_____meses ( )AMEP_____meses

7. Uso de contraceptivo hormonal: ( ) Oral ( ) Vaginal ( ) Adesivo ( ) Outro
Início: _____ Término:_____ Tempo:_____
8.Idade da menopausa: ( ) SIM _____ anos. ( ) NÃO
9.Causa da menopausa: ( )Espontânea.
( )Radiação. ( )Histerectomia ou retirada dos ovários.
10. Terapia de reposição hormonal:
( ) Sim ( ) Não
Nome comercial: _____Tipo: (J)Conjugado (NJ) Não conjugado
Administração: (O) Oral (V) Vaginal (A) Adesivo ( ) Outro
Início: _____ Término:_____ Tempo:_____
11. Utiliza(ou) algum medicamento por longo prazo? ( ) SIM ( ) NÃO
Qual?_____ Indicação:_____
Início do uso: _____ Término do uso:_____
12. Já fez alguma mamografia?
(S) Sim (N) Não
Com que idade fez a primeira mamografia?_____ anos.
Quando foi a sua última mamografia?_____ _____
Desde a primeira mamografia com que frequência fez as outras?
(1) de 6 em 6 meses.
(2) Anualmente.
(3) 1 vez a cada 2 anos.
(4) 1 vez a cada 3 anos.
(5)1 vez a cada 4-5 anos.
(6) 1 vez a cada 6-10 anos.
(7) fez menos frequentemente que a cada10 anos.
(8) Só fez uma vez.

C.ATIVIDADE FÍSICA (AGITA/2002-INCA/2002)

Em quantos dias da semana há pelo menos 10 minutos seguidos de atividade leve- aquela na qual não há aumento dos batimentos cardíacos?_____
Quanto tempo gasta se exercitando? _____

Em quantos dias da semana há pelo menos 10 minutos seguidos de atividade moderada e intensa- aquela na qual há aumento dos batimentos cardíacos?_____
Quanto tempo gasta se exercitando?_____

D.HISTÓRIA DA DOENÇA

1.História prévia de lesão benigna na mama? ( )Sim ( )Não
2. História familiar de câncer de mama? ( ) Não há casos
( ) Sim há casos. Quem? ( )Avó ( )Mãe ( )Tia ( )Filha ( )Irmã
3. História pessoal de diabetes? ( )Sim ( )Não
4. História pessoal de gota? ( )Sim ( )Não
5. História pessoal de Insuficiência renal?? ( )Sim ( )Não

E.ANTROPOMETRIA

Peso atual: _____ Peso usual: _____
Altura: _____ IMC: _____
%GC:_____
Peso aos 18 anos:_____ IMC aos 18 anos: _____

Circunferência cintura: _____ Circunferência quadril: _____

Ganho de peso? ( ) SIM ( )NÃO Quantos quilos?_____Quando?_____
Perda de peso?( ) SIM ( )NÃO Quantos quilos?_____ Quando?_____

G.BEBIDAS ALCOÓLICAS

( )Sim ( ) Não ( ) Já utilizou.

Bebida	Início	Término	Tempo	Quantidade/Recipiente	Frequência
Pinga				( ) copo ( ) garrafa	( )dia( )sem.( )mês
Cerveja				( ) copo ( ) garrafa ( ) lata	( )dia( )sem.( )mês
Martini				( ) copo ( ) garrafa	( )dia( )sem.( )mês
Campari				( ) copo ( ) garrafa	( )dia( )sem.( )mês
Vinho				( ) copo ( ) garrafa	( )dia( )sem.( )mês
Outros				( ) copo ( ) garrafa	( )dia( )sem.( )mês

G.FUMA? ( )Sim ( ) Não Tempo?_____
Cigarros/dia?_____ Tipo cigarro: ( ) Filtro ( ) sem filtro ( ) Fumo/rolo

Já fumou? ( )Sim ( ) Não Início:_____ Término:_____
Cigarros/dia?_____ Tipo cigarro: ( ) Filtro ( ) sem filtro ( ) Fumo/rolo

Anexo 4: Questionário semiquantitativo de frequência alimentar

CARNES EM GERAL	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Bacon							?Costumava comprar carne com gordura? :
Costelinha suína							( ) Sim ( ) Não
Frango assado							
Frango frito							
Carne bovina cozida							?Costumava retirar a gordura da carne para comer? :
Carne bovina grelhada							( ) Sim ( ) Não
Carne bovina frita							
Carne bovina moída							
Carne suína cozida							
Carne suína grelhada							
Carne suína frita							?Costumava retirar a pele para comer? :
Torresmo							( ) Sim ( ) Não
Peixe frito							
Peixe ensopado							
Peixe empanado							?Com que frequência frequentava churrascos? :
Lingüiça ( ) suína ( ) frango							( ) VEZES ( ) mês( ) trim ( ) raral/( ) nunca
Hamburguer / Steak / Nuggets							
Almôndega ( ) bovina ( ) frango							
Salame							?Acondicionava as carnes coccionadas
Mortadela/Pres./Apresuntado							em latas de gordura? ( ) sim ( ) não
Miúdo fígado ( ) boi ( ) frango							
Miúdos moela							
Miúdos coração ( ) boi ( ) frango							
Sardinha enlatada ( ) c/ ( ) s/ óleo							
Atum sem óleo ( ) c/ ( ) s/ óleo							
Salsicha hot dog							
Ovo de galinha frito							
Ovo de galinha cozido							
Omelete							
Chouriço							

LEITE E DERIVADOS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Leite integral							
Leite desnatado							
Iogurte/Coalhada							
Extrato solúvel de soja							?Costumava retirar a nata do leite para beber? :
Queijo minas frescal							( ) Sim ( ) Não
Queijo prato/mussarela							
Requeijão cremoso							
LIPÍDIOS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Margarina ( ) comum ( ) light ( ) c/ ( ) s/ sal							Produto utilizado na cocção?:
Manteiga ( ) c/ ( ) s/ sal							( )óleo( )gordura( ) banha( )gordura hidrogenada
Nata							
LEGUMINOSAS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Feijão simples							
Feijoada							
Soja cozida							
Ervilha enlatada							
Ervilha vagem/lentilha/grão de bico							
Amendoim							
SALGADINHOS E OUTROS INDUSTRIALIZADOS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Coxinha frita							
Pastel frito							
Quibe frito							
Empadinha assada							
Pizza							
Pipoca							
Chip's (batatas e outros)							
Sopa industrializada							
Achocolatado							
Café							
Chá mate							
Chá preto							
Chá verde							
Refrigerante							
Molho inglês							
Molho soja(Shoyo)							
Molho pimenta							
Ketchup							
Mostarda							

PÃES E SIMILARES	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Biscoito de polvilho frito							
Biscoito de polvilho assado							
Biscoito papa ovo							
Biscoito de nata							
Biscoito amanteigado							
Biscoito recheado industrial							
Biscoito água e sal							
Biscoito doce							
Pão francês							
Pão de forma							
Pão integral							
Pão de queijo							
Bolo simples							
Broa de fubá							
CEREAIS E FARNÁCEOS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Arroz cozido							
Angu							
Macarrão							
Farinha de mandioca							
Farinha de milho							
Canjiquinha							
Bambá de couve							
SOBREMESA	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Cajuzinho							
Gelatina							
Pudim							
Doce de leite em pasta							
Chocolate/Bombons							
Goiabada							
Doce de fruta							Utiliza adoçante? ( ) Sim ( ) Não
Balas/pirulitos/chicletes							Marca:_____
Ambrosia/quindim							
Sorvete							

FRUTA A,B,C	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Abacate							
Abacaxi							
Banana							
Castanha							
Laranja							
Maçã							
Mamão							
Melão							
Manga							
Uva							
SUCOS	MEDIDAS CASEIRAS	DIÁRIA	SEMANAL	QUINZENAL	MENSAL	NUNCA OU RARAMENTE	
Suco natural sabor _____							
Suco industrializado sabor _____							
Suco (pó) sabor _____							

CONSUMO FAMILIAR MENSAL OU SEMANAL

ALIMENTO	QUANTIDADE		Nº pessoas q consomem	Consumo individual
	Mensal	Semanal		
1.Leite condensado				
2.Creme de leite				
3. Maionese				
4.Açúcar				
5.Oleo de _____				
6.Gordura hidrogenada/banha				
7.Azeite				
8.Alho				
9.cebola				
10.Caldo Knorr/Arisco				
11.Pasta de alho e sal				
12.Sal				

Somatório dos alimentos cozidos (+ 1mL óleo): _____

Somatório dos alimentos fritos (+ 2mL óleo): _____

Total de óleo adicionado: _____

Número de pessoas que fazem a maior parte das refeições em casa? _____
Mudança nos hábitos alimentares nos últimos 5 anos? ( ) SIM ( ) Não
Motivo (em caso afirmativo):_____