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# HISTÓRIA FAMILIAR DE CÂNCER, EXPOSIÇÃO MATERNA A MEDICAMENTOS E O DESENVOLVIMENTO DE LEUCEMIA EM MENORES DE 2 ANOS

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Orientador

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Tese apresentada ao Programa de Saúde Pública e Meio Ambiente da Escola Nacional de Saúde Pública Sérgio Arouca como parte dos requisitos para a obtenção do título de Doutor em Ciências.

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*“A mente que se abre a uma nova idéia jamais voltará ao seu tamanho original”*

**Albert Einstein**

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## 1. Introdução

O câncer infantil é uma doença rara, representada por menos de 1% de todas as neoplasias diagnosticadas nos países em desenvolvimento **(Reis *et al.* 2007)**. A incidência anual do câncer infantil pode variar de 70 a 160 casos por milhão de habitantes menores de 15 anos em todo o mundo **(Stiller, 2004).**

Dentre as diferentes neoplasias infantis, as leucemias constituem as mais frequentes na infância, representando cerca de 30% de todos os diagnósticos de neoplasias malignas **(Pui *et al.* 2004)**. Dentre os subtipos de leucemias na infância, a Leucemia Linfocítica Aguda (LLA) é a mais frequente, encontrada em 75% dos casos pediátricos **(Pui *et al.* 2004)**. Trata-se de uma neoplasia maligna, caracterizada pelo acúmulo de células linfoides imaturas na medula óssea, levando a infiltração nos tecidos por células leucêmicas **(Kebraei *et al.* 2003)**.

O diagnóstico definitivo do tipo leucêmico é realizado pelo exame de medula óssea, baseado na caracterização morfológica, imunofenotípica e citogenética dos blastos leucêmicos. Os tipos de células leucêmicas vão determinar a progressão da doença e, consequentemente, o curso clínico da mesma **(Pui, 1998)**.

O aumento observado na incidência de neoplasias, especialmente as leucemias e os tumores do sistema nervoso central (SNC), é parcialmente decorrente do desenvolvimento e aprimoramento de métodos de diagnóstico, como o avanço na utilização de técnicas de biologia molecular, possibilitando assim a detecção de anormalidades genéticas **(Braga *et al.* 2002)**. Diante da melhoria no diagnóstico das leucemias, é observado também um progresso terapêutico e, consequentemente, uma melhora nos resultados de sobrevivência em cinco anos das crianças afetadas **(De Camargo, 2003; Howard *et al.* 2004)**.

Além disso, em diversos países observa-se uma redução nas taxas de mortalidade por câncer infantil. Nos EUA, a taxa de mortalidade por leucemia infantil declinou cerca de 50% entre os anos de 1975 e 1995, com uma diminuição estatisticamente significativa de 3,4% ao ano **(Ries *et al.* 1999)**. No Brasil, estudos de séries temporais têm apresentado resultados semelhantes de redução na mortalidade por leucemia em menores de 15 anos **(Braga *et al.* 2002; Couto *et al.* 2010)**.

A avaliação da incidência dos subtipos de leucemias no mundo tem apresentado variações importantes em relação à distribuição geográfica, sexo, faixa etária e grupos étnicos, sugerindo o envolvimento de diferentes fatores etiológicos no processo de leucemogênese **(Greave, 1999)**. Neste sentido, acredita-se que seja improvável que a etiologia da leucemia seja resultante de único evento, mas sim da interação entre fatores ambientais e genéticos **(Greaves, 1999; Pombo-de-Oliveira *et al.* 2006)**.

Adicionalmente, acredita-se que os estudos de leucemia infantil sejam os mais indicados para a avaliação das exposições ambientais, devido ao período de latência reduzido desta neoplasia, favorecendo assim investigações com foco na exposição materna no período gestacional **(Pombo-de-Oliveira *et al.* 2006)**. Diante disso, considerando a suscetibilidade do concepto a diversos fatores de exposição e as evidências de que a etiologia da leucemia seja decorrente da interação entre fatores ambientais e genéticos, torna-se oportuno avaliar a contribuição dos fatores hereditários e a exposição a medicamentos no desenvolvimento de leucemia aguda infantil.

## 2. Referencial teórico

### 2.1. O sistema hematopoiético e as leucemias

O processo de diferenciação, proliferação e liberação das células sanguíneas para exercerem suas funções biológicas no organismo é denominado de hematopoiese. Este processo é comandado por uma célula precursora hematopoiética denominada *stem cell* **(Figura 1)**. Este fenômeno começa durante o período intra-uterino e ocorre por toda a vida, devido ao curto período de vida celular e a importância vital das células sanguíneas **(Bondurant & Koury, 2004)**. Além disso, este processo é regulado, de modo que as proporções celulares de cada elemento permaneçam relativamente constantes em estados de normalidade, sendo que, para isso, existem fatores em cada linhagem celular controlando a produção **(Bondurant & Koury, 2004)**.

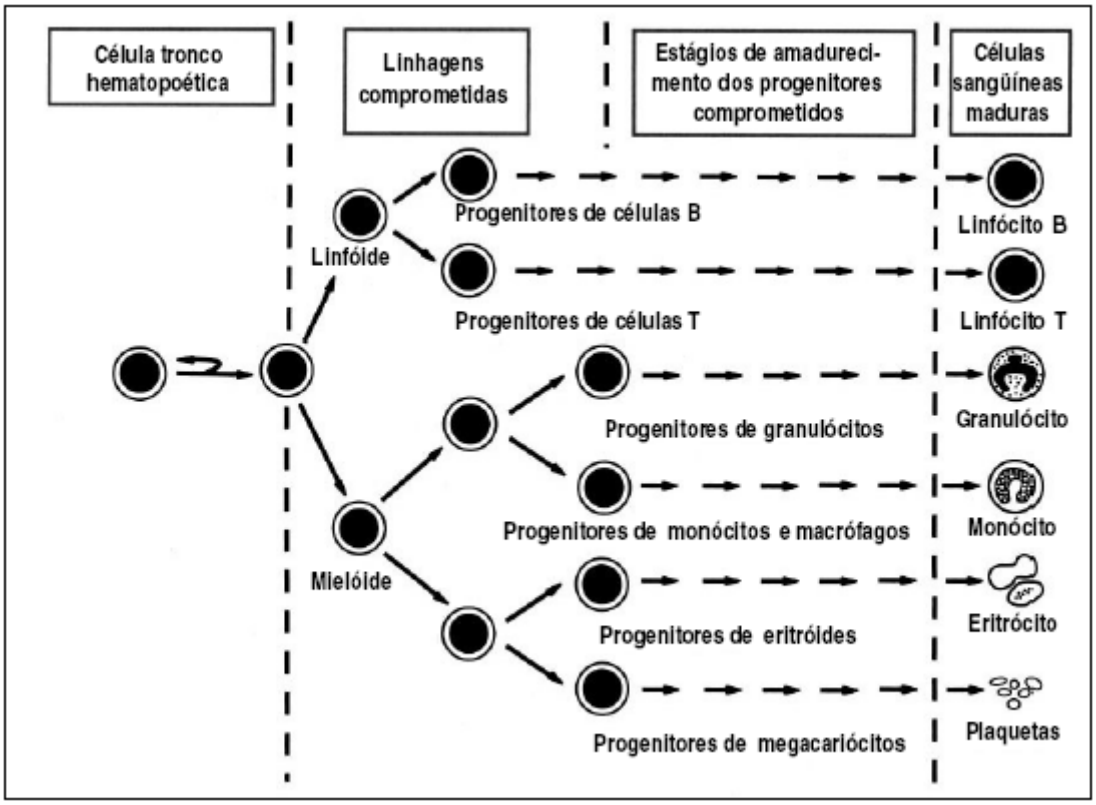


**Figura 1:** Diagrama de formação celular pelo sistema hematopoiético

Durante o estágio de formação celular, podem ocorrer alterações na célula progenitora que provocam perdas na diferenciação e proliferação normal, ocasionando no processo de leucemogênese **(Greaves, 1999)**. Este processo resulta no desenvolvimento de uma expansão clonal maligna decorrente de alterações genéticas no precursor hematopoiético, levando ao acúmulo de células imaturas na medula óssea e posteriormente no sangue periférico **(Bondurant & Koury, 2004)**. De maneira semelhante a outras neoplasias, tais alterações genéticas envolvem genes com função no controle do ciclo celular, denominados oncogenes e antioncogenes **(Greaves, 2003).**

## 2.2. Classificação das leucemias

As leucemias são classificadas a partir de dois fatores principais, pelo tipo de célula afetada e pela rapidez do desenvolvimento e progressão da doença **(Pui *et al.* 2004)**. No primeiro caso, as leucemias são resultantes da transformação do progenitor celular, comprometendo assim as linhagens denominadas linfoide ou mieloide. Esta classificação é definida após o diagnóstico final realizado por três metodologias distintas (morfologia, imunofenotipagem e citogenética-molecular) que são recomendadas em conjunto pela Organização Mundial de Saúde (OMS).

As leucemias podem ser classificadas também, de acordo com a fisiopatologia da doença, nas formas agudas ou crônicas, sendo que nas leucemias agudas as células leucêmicas pertencem ao grupo de células progenitoras mais imaturo, enquanto que nas leucemias crônicas as células malignas se desenvolvem em estágios de maturação mais tardios **(Kersey, 1997)**.

O principal tipo leucêmico na infância é a Leucemia Linfóide Aguda (LLA) representada por aproximadamente 75% dos casos **(Pui *et al.* 2004)**, seguida pela

Leucemia Mielóide Aguda (LMA) presente em 15-20% dos casos pediátricos **(Hall, 2001)**.

### 2.2.1. Leucemia Linfóide Aguda (LLA)

A LLA é o tipo mais comum de câncer infantil, constituindo cerca de um terço de todas as neoplasias malignas da criança, sendo mais frequente em crianças brancas e no sexo masculino **(Gurney *et al.* 1995)**.

A classificação da LLA baseada na morfologia celular apresenta três subtipos distintos, denominados $L_1$, $L_2$ e $L_3$ (FAB: *French American British group*). O subtipo $L_1$ é o mais frequente, sendo caracterizado por blastos pequenos e um aumento da relação núcleo/citoplasma. Por outro lado, o subtipo $L_2$ apresenta blastos maiores, mas não uniformes, com nucléolos proeminentes e uma relação núcleo/citoplasma menor. Enquanto que o subtipo $L_3$ é a forma mais rara da doença e de pior prognóstico, caracterizada por uma intensa vacuolização citoplasmática **(Lopes *et al.* 2000)**.

Considerando a classificação pela metodologia de imunofenotipagem, utilizando a técnica de citometria de fluxo, as leucemias são divididas por meio da positividade de marcadores específicos, podendo ser classificadas em pré-B, B e T. Além destes marcadores, os blastos linfóides podem ser caracterizados por marcadores de membrana como CD7, CD10, CD19 e CD20 e por marcadores citoplasmáticos com TdT **(Lopes *et al.* 2000)**. A análise citogenética permite observar alterações pelo número cromossomal, translocações, inversões e deleções gênicas **(Greaves, 1999; Lopes *et al.* 2000)**.

A finalidade destas classificações é distinguir as leucemias linfóides das mielóides, principalmente quando os blastos são muito indiferenciados. Além disso,

estas classificações podem também determinar o prognóstico das leucemias **(Cavalcanti Jr, 1997)**.

### 2.2.2. Leucemia Mielóide Aguda (LMA)

A LMA é uma doença predominantemente presente em idosos (acima de 60 anos de idade), representando mais de 50% dos casos nesta faixa etária **(Iovino & Camacho, 2003; Rodrigues *et al.* 2003)**, sendo mais comum no sexo masculino **(Bain, 2003)**. Entretanto, a LMA representa uma parcela importante de casos pediátricos, com cerca de 15-20% das leucemias agudas enquanto nos adultos esta proporção pode chegar a 80% dos doentes **(Hall, 2001; Martins & falcão, 2000).** A LMA apresenta um prognóstico mais grave, especialmente em pacientes idosos **(Bene *et al.* 1999)**.

De acordo com a classificação da FAB, a análise morfológica da LMA apresenta oito subtipos:

M0 – LMA sem diferenciação morfológica;

M1 – LMA com mínima diferenciação morfológica;

M2 – LMA com diferenciação (componente monocítico < 20%);

M3 – LMA promielocítica hipergranular ou M3 variante hipogranular;

M4 – LMA mielomonocítica (células monocíticas ≥ 20%) ou M4 variante;

M5 – LMA monocítica (com células monocíticas ≥ 20% das células leucêmicas)

M5a – LMA monoblástica (sem diferenciação, blastos ≥ 80%)

M5b – LMA monocítica (com diferenciação, blastos < 80%);

M6 – eritroleucemia e variante;

M7 – LMA megacarioblástica.

Entretanto, essa classificação morfológica é questionada pela OMS, pois não utiliza os demais critérios de diagnóstico (imunológicos e citogenéticos).

A classificação da LMA pela imunofenotipagem é importante para distinção das linhagens linfóide e mielóide, além do monitoramento do tratamento para detecção de doença residual mínima. Neste sentido, a análise pela imunofenotipagem consiste, principalmente, no diagnóstico das LMA M0 e M7, além de auxiliar em alguns casos de M5a e no diagnóstico das LMA M3, M2 e M1/M2 **(Martins & Falcão, 2000; Bain, 2003)**. Entre os principais marcadores de membrana estão o CD13, CD33, CD34 e CD117 **(Swirsky & Richards, 2001; Wang *et al.* 2005)**.

Os estudos moleculares são importantes na detecção de anormalidades no clone leucêmico, auxiliando na precisão do diagnóstico e no prognóstico da doença **(Swirsky & Richards, 2001; Hall, 2001)**. As aberrações citogenéticas adquiridas são detectadas em 55-75% dos casos de LMA diagnosticados recentemente **(Grimwade, 2001; Lowenb, 2001)**. A análise molecular é importante na identificação de uma recombinação molecular característica de um determinado tipo de LMA, facilitando a diferenciação dos oito subtipos conhecidos **(Taylor & Stasi, 1996; Leroy *et. al*, 2005)**.

## 2.3. Tratamento das leucemias

O tratamento das leucemias, principalmente em crianças, tem apresentado resultados satisfatórios, sendo considerada a neoplasia com maior sucesso terapêutico em menores de 15 anos **(Pui *et. al*, 2003)**. O primeiro agente efetivo no tratamento da LLA foi um antifolato (aminopterina), baseado no conhecimento de que o acido fólico era essencial para a hematopoiese normal. Entretanto, somente em 1948, o médico Sidney Farber descreveu o efeito do tratamento com aminopterina em 50 crianças com LLA, observando aumento da sobrevida em mais de cinco anos **(Farber *et. al*, 1948)**.

Nos anos seguintes, novos fármacos foram testados como os corticosteróides (1950), antipurinas (1953) e vincristine (1963). Durante o tratamento nos anos 60, o uso

de drogas combinadas apresentou sucesso na tentativa de remissão da doença, porém, não era considerado o somatório dos efeitos adversos. Neste período, surge o protocolo *Total therapy* com percentual de cura de 50% com seguimento por mais de 30 anos **(Aur *et. al*, 1971)**.

Nos últimos 25 anos, diversos grupos e instituições têm sugerido aumento no percentual de cura com índices de até 80%, resultando em taxas de mortalidade reduzidas **(Braga *et. al*, 2002; Pui *et. al*, 2003; Couto *et. al*, 2010)**. A maioria dos esquemas terapêuticos modernos é constituída de cinco fases: indução da remissão da doença, intensificação-consolidação, reindução, prevenção da leucemia no sistema nervoso central (SNC) e continuação ou manutenção da remissão **(Pedrosa & Lins, 2002)**.

A LLA evoluiu de uma neoplasia mal definida e intratável na metade do século passado para uma doença bem relatada na literatura e com resultados satisfatórios de cura no início deste século **(Pedrosa & Lins, 2002)**. Atualmente, a identificação de vários fatores prognósticos permite a estratificação dos pacientes em grupos de risco, possibilitando, assim, uma abordagem terapêutica diferenciada. Neste sentido, os grupos de maior risco são tratados com terapias mais intensas, cada vez mais eficazes, enquanto os grupos de baixo risco apresentam melhor sobrevida, podendo ser poupados de efeitos deletérios devido ao tratamento **(Souza & Coelho, 1998; Felix, 2000)**.

O desafio de tornar a LLA uma doença curável começa a ser alcançado ao se observar sobrevida maior que 50% em países com recursos limitados, como por exemplo, em El Salvador **(Bonilla *et al.* 2000)**. Estudos brasileiros também têm observado aumento na sobrevida da leucemia infantil. Em Recife, a sobrevida global em cinco anos passou de 32% (1980-1989) para 63% (1997-2002), sendo que no primeiro período houve uma recaída de 14% e 16% de abandono ao tratamento, enquanto que no último período avaliado a recaída foi de apenas 3,3% e o abandono de 0,5% **(Howard *et***

***al.* 2004)**. No Hospital do Câncer de São Paulo também foi observada melhoria na sobrevida em cinco anos, passando de 13% (1975-1979) para 55% (1995-1999) **(De Camargo, 2003)**.

O tratamento da LMA consta basicamente de quimioterapia, porém, é realizado também o transplante de medula óssea em casos de prognóstico desfavorável e nas recidivas. O tratamento divide-se em indução de remissão e pós-remissão, podendo haver diferenças na terapêutica de acordo com a faixa etária ou subtipos específicos **(Greer & Baer, 2004)**.

## 2.4. Fatores ambientais associados à leucemia infantil

Estudos epidemiológicos têm descrito vários possíveis fatores de risco para as leucemias na infância com objetivo de entender sua etiologia. Greaves (1999) propôs um modelo dos possíveis fatores causais, divididos em alterações genéticas, fatores ambientais e modificações adquiridas.

Os fatores ambientais apresentados a seguir incluem possíveis associações entre a exposição peri-gestacional e o desenvolvimento de leucemias na infância.

### 2.4.1. Radiações ionizantes

Entre os fatores ambientais associados ao desenvolvimento de leucemia infantil, apenas a exposição a radiações ionizantes é reconhecida como fator de risco, enquanto que os demais apresentam resultados controversos **(Belson *et al.* 2007)**. Um estudo realizado no Japão observou aumento na estimativa de risco (OR=4,7; IC95%:1,2-19,9) entre a exposição a campos magnéticos em níveis elevados (>0,4μT) e o

desenvolvimento de LLA infantil quando comparado ao grupo de referência (<0,1 μT) **(Kabuto *et al.* 2006)**.

Calvente e colaboradores (2010) realizaram uma revisão sistemática e sugeriram que as evidencias epidemiológicas apontam que a exposição a radiações ionizantes estão associadas ao aumento na incidência de leucemia em crianças expostas a campos magnéticos de baixa freqüência.

Além disso, é importante considerar que a exposição de crianças a radiação (incluindo a não-ionizante) tem sido implicada a diferentes patologias, despertando preocupação sobre o aumento exagerado de uso de telefones celulares e outras fontes de radiação não-ionizante. Os estudos que avaliam as exposições a radiações em crianças são importantes, por se tratar de indivíduos mais vulneráveis do que os adultos, merecendo atenção especial de pesquisa **(Ramon *et al.* 2005)**.

Neste contexto, de acordo com a Agência Internacional de Pesquisa em Câncer (IARC), os campos magnéticos de baixa freqüência são classificados como possíveis carcinogênicos para os humanos (classe 2b) **(IARC, 2002)**.

### 2.4.2. Alimentação

A alimentação é um fator de exposição muito importante durante a gestação, porém, existem poucos estudos abordando a associação entre esta exposição e o desenvolvimento de leucemia infantil. Um estudo caso-controle realizado na Grécia com crianças menores de 5 anos de idade, observou que a ingestão de vegetais (OR=0,76; IC95%:0,60-0,95), frutas (OR=0,72; IC95%:0,57-0,91), peixes e frutos do mar (OR=0,72; IC95%:0,59-0,89) estava associada ao efeito protetor de desenvolver LLA. Enquanto que analisando o consumo de carnes (OR=1,25; IC95%:1,00-1,57) e

açúcares (OR=1,32; IC95%:1,05-1,67) durante a gestação houve um discreto aumento no risco de desenvolver LLA **(Petridou *et al.* 2005)**.

Um estudo realizado nos EUA também observou efeito protetor entre o consumo materno durante a gestação de vegetais (OR=0,65; IC95%:0,50-0,84), frutas (OR=0,81; IC95%:0,65-1,00), fontes de proteínas (OR=0,55; IC95%:0,32-0,96) e o consumo de leguminosas (OR=0,81; IC95%:0,65-1,00) e o desenvolvimento de leucemia em menores de 2 anos **(Kwan *et al*, 2009).**

Estes resultados são relevantes para sustentar a hipótese da etiologia da leucemia ainda no período gestacional, visto que a exposição fetal a compostos bioativos presentes em vegetais, frutas, alimentos protéicos, vitaminas e minerais podem contribuir na redução de risco de desenvolver diferentes tipos de neoplasias **(Greenwald *et al*, 2001; Collins *et al*, 2003).**

### 2.4.3. Tabagismo e etilismo

Os hábitos de vida durante a gestação, como tabagismo e etilismo, associados ao desenvolvimento de leucemia infantil já foram avaliados em diferentes estudos, apresentando divergências nos resultados **(Brondum *et al.* 1999; Chang *et al.* 2006; Milne *et al.* 2012).**

Um estudo australiano observou associação positiva entre o hábito de tabagismo paterno e o desenvolvimento de LLA na infância (OR=1,35 IC95%:0,98-1,86), enquanto que para exposição materna não foram encontradas estimativas estatisticamente significativas **(Milne *et al.* 2012).** Outros estudos observaram, também, um discreto aumento nas estimativas de risco, sem significância estatística, entre o tabagismo materno durante a gravidez e o desenvolvimento de LLA na infância **(Chang *et al.* 2006; McArthur *et al.* 2008)**.

A mesma divergência de resultados é observada entre o consumo de álcool durante a gestação e o risco no desenvolvimento de leucemia. Shu e colaboradores (1996) observaram uma associação positiva para LMA (OR=2,6 e IC95%:1,4-5,1) e para LLA (OR=1,43 e IC95%:1,0-2,4). Assim como Menegaux e colaboradores (2007) também observaram aumento nas estimativas de risco, analisando o consumo de bebidas alcoólicas em doses superiores a um copo por dia e o desenvolvimento de LLA na infância (OR=2,8 IC95%:1,3-5,9). Entretanto, outro estudo realizado nos EUA observou efeito protetor (OR=0,64; IC95%:0,43-0,94) entre o consumo de bebidas alcoólicas durante a gestação e o desenvolvimento de leucemia em lactentes **(Slater *et al.* 2011)**.

### 2.4.4. Solventes orgânicos

A Agência Internacional de Pesquisa em Câncer tem identificado vários compostos químicos sendo comprovadamente fatores causais de câncer (grupo 1), tais como benzeno, cádmio e sílica. Outras substâncias químicas cujas evidências na carcinogênese são ainda objeto de confirmação, são denominadas como agentes provavelmente (grupo 2A) ou possivelmente (grupo 2B) associadas ao desenvolvimento de neoplasias **(IARC, 1987)**.

Entre os agentes químicos com potenciais efeitos carcinogênicos estão os solventes orgânicos. Em uma revisão sobre estes solventes e diversas neoplasias, foi observado um aumento nas estimativas de risco e o desenvolvimento de câncer com solventes específicos, como benzeno, tolueno, 1,1-tricloretano e metileno cloreto **(Lynge *et al.* 1999)**. Embora o benzeno seja reconhecido como agente leucêmico pela IARC, os demais solventes orgânicos não são classificados como cancerígenos por

agências reguladoras, favorecendo a exposição de trabalhadores em diversos setores industriais.

Neste sentido, um estudo caso-controle de base populacional foi conduzido no Canadá para avaliar a exposição ocupacional materna a solvente orgânico durante a gestação e o desenvolvimento de leucemia infantil. Foi observado aumento nas estimativas de risco para exposição a substâncias específicas, como tolueno (OR=1,88, IC95%:1,01-3,47), alcanos (OR=1,78, IC95%:1,11-2,86) e hidrocarbonetos aromáticos mononucleares (OR=1,64, IC95%:1,12-2,41) e o desenvolvimento de leucemia em crianças menores de 14 anos **(Infante-Rivard *et al.* 2005)**.

### 2.4.5. Peso ao nascer

O peso ao nascer é um fator de risco para leucemia na infância bem relatado na literatura nacional e internacional. Um estudo caso-controle brasileiro observou uma associação positiva (OR=2,28; IC95%:1,08-4,75) entre peso ao nascer elevado (>3999g) e o desenvolvimento de leucemia aguda no lactente, após controlar por variáveis de confundimento **(Koifman *et al.* 2008)**.

Um estudo de metanálise, incluindo 32 trabalhos, sugeriu associação positiva, estatisticamente significativa, entre peso ao nascer e risco de desenvolver leucemia, observando que alto peso estava associado tanto a LLA quanto a LMA. Neste mesmo estudo foi proposto que baixo peso ao nascer estava associado apenas com LMA, sugerindo uma função em forma de U, ou seja, um aumento nas estimativas de risco para crianças com baixo ou elevado peso (menores de 2500g e maiores de 3999g) **(Caughey & Michels, 2009)**.

Outros mecanismos sugeridos para o desenvolvimento de leucemia em lactentes associados ao peso ao nascer seriam as fusões gênicas (principalmente os rearranjos do

gene *MLL*), alterações nas quais representam cerca de 80% dos casos de leucemia em menores de 1 ano **(Ross *et al.* 1996)**. Nessa perspectiva os estudos moleculares têm sugerido que o aumento na expressão do gene *IGF-1* (*insulina-like growth factor-1*) promove a proliferação de células danificadas, visto que, o aumento do peso ocasiona em maior quantidade de células precursoras da linhagem sanguínea (*steam cell*), que por consequência, aumenta a quantidade de células imaturas expostas a eventos nocivos, tornando-se mais susceptíveis à transformação maligna **(Boyne *et al,* 2003).**

### 2.4.6. Medicamentos

O uso de medicamentos antes ou durante a gravidez continua sendo um problema de saúde pública, sendo que o uso materno no período gestacional pode levar ao desenvolvimento de mal formações congênitas, baixo peso ao nascer e atrasos físicos no desenvolvimento da criança **(Khera, 1987; Broekhuizen *et al.* 1992, D'Apólito, 1998)**. Diversos estudos sustentam a hipótese sobre a etiologia da leucemia no período intrauterino, enfatizando os agravos da exposição por agentes tóxicos ao feto no início da gravidez **(Ford *et al.* 1997; Greaves, 1999; Pombo-de-Oliveira *et al*. 2006)**.

Os analgésicos são uma das classes mais prescritas entre os medicamentos consumidos na gestação. São fármacos adquiridos facilmente sem prescrição médica, sendo utilizados de forma indevida na maioria das vezes **(Glover *et al.*, 2003).** Grande parte dos analgésicos pertence a uma classe de fármacos denominada NSAIDs (N*on-Stereoidal Antiinflamatory Drugs)*, que atuam na inibição das enzimas COXs (Ciclooxigenases) **(Burdan *et al*, 2006).** O uso de fármacos inibidores de COXs durante a gestação esteve associado à prematuridade, ao aborto espontâneo e às mal formações congênitas, como alterações cardíacas e diafragmáticas **(Ostensen & Skomsvoll, 2004).** O paracetamol, ao contrário da dipirona e acido acetilsalicílico, é um inibidor seletivo

da COX-3, sem efeito antiinflamatório. Nas últimas décadas, acreditava-se que o paracetamol era o antipirético e analgésico mais seguro para ser consumido durante a gestação **(Graham & Scott, 2005).**

É importante considerar que a biotransformação de fármacos é uma etapa primordial no processo de eliminação e diminuição da toxicidade de substâncias químicas. Além disso, pode ser também responsável pela formação de metabólitos reativos intermediários que se ligam a macromoléculas do organismo, como a molécula de DNA. Dependendo da estrutura e do tipo de ligação, diferentes efeitos patológicos podem ocorrer, inclusive com o feto em desenvolvimento, como mutagênese, carcinogênese e teratogênese **(Oshima-Franco & Franco, 2003).** Neste sentido, é regra geral que qualquer medicamento consumido no período gestacional deve ser ingerido em baixas doses terapêuticas, reduzindo assim o potencial risco de alterações adversas no desenvolvimento do embrião **(Ostensen & Skomsvoll, 2004).**

Um estudo realizado nos EUA observou efeito protetor limítrofe entre o uso de NSAIDs (OR=0.60; 95% C.I. 0.37-0.97) e paracetamol (OR=0.66; 95% C.I. 0.43-1.01) e o desenvolvimento de LMA. Porém, para o uso de aspirina não foi observado significância estatística (OR=0.55; 95% C.I. 0.24-1.26). Por outro lado, Pombo-de-Oliveira e colaboradores (2006) observaram aumento nas estimativas de risco para o uso de dipirona (OR=1,45; IC95%:1,02-2,06) e de pílulas esteróides (OR=8,76; IC95%:2,85-26,93) durante a gestação e o desenvolvimento de leucemia no lactente. O consumo materno de dipirona durante a gravidez esteve associado também ao desenvolvimento do tumor de Wilms (OR=10,9; IC95%:2,4-50,0) **(Sharpe & Franco, 1996).**

Em relação aos fármacos que apresentam possíveis efeitos de proteção no período gestacional, as vitaminas e a suplementação mineral (sulfato ferroso e o ácido fólico) são as mais estudadas. Porém, poucos estudos relatam seus efeitos no

desenvolvimento de leucemia infantil **(Ross *et al.,* 2005; Kwan *et al.,* 2007; Goh *et al.* 2007).** Apesar dos resultados desses estudos serem inconclusivos, existe uma plausibilidade biológica suportando a hipótese de efeito protetor entre a exposição a vitaminas e suplementação mineral e o risco de desenvolver diferentes neoplasias. Ames (2001) sugere que a ingestão inadequada de importantes micronutrientes pode contribuir para os danos na molécula de DNA, podendo ocasionar lesões oxidativas que, por sua vez, podem contribuir para o desenvolvimento de neoplasias. Neste sentido, Kim (1999) sugere que os mecanismos biológicos envolvidos nos efeitos de proteção ao desenvolvimento de diferentes neoplasias seriam alterações na metilação e reparo de DNA.

Thompson e colaboradores (2001) observaram efeito protetor entre o uso de suplementos de ferro ou acido fólico e o desenvolvimento de LLA em menores de 14 anos (OR=0.37 95% C.I. 0.21-0.65). Estes autores avaliaram o consumo exclusivo de ferro, mas não foram observadas estimativas de risco estatisticamente significativas (OR=0.75 95% C.I. 0.37-1.51).

## 2.5. Fatores genéticos associados à leucemia infantil

O advento da epidemiologia molecular tem sido fundamental em trabalhos envolvendo o mapeamento de genes suscetíveis ao desenvolvimento de diversas doenças. O acúmulo de alterações genéticas em genes que regulam o processo de divisão celular leva à aquisição de um fenótipo maligno em que as células adquirem vantagem de sobrevida e deixam de exercer suas funções normais **(Sinnett *et al*, 2000)**. Sendo assim, a análise de clones leucêmicos pode fornecer informações fundamentais sobre a transformação maligna e quais são os mecanismos moleculares envolvidos **(Pui *et al*, 2004)**.

Os estudos das aberrações cromossômicas recorrentes e específicas, e sua associação a subtipos de leucemia definidos por linhagem, tipo celular e estágio de diferenciação, possibilitam identificar os genes que estão envolvidos na perda do controle dos mecanismos reguladores do ciclo celular e na aquisição do fenótipo maligno **(Mittelmann *et al*, 1997)**.

Diversos genes estão ligados ao desenvolvimento de câncer, entre os quais, muitos são polimórficos. Estes genes podem apresentar substituição de uma única base de um nucleotídeo ou uma variação no número de pequenas sequências repetitivas de DNA. Estas variações podem influenciar a taxa de transcrito gênico e consequentemente a quantidade e atividade das enzimas, prejudicando o bom funcionamento do organismo **(Witkin *et al.*, 2002)**.

Um marco na pediatria oncológica foi a descoberta das modificações cromossômicas envolvendo alguns genes que, uma vez alterados, favoreciam o desenvolvimento de diversas neoplasias, entre elas, as leucemias **(Greaves, 2003)**. Além disso, estudos têm sugerido que a exposição a agentes tóxicos durante o período intrauterino pode levar a uma série de agravos ao desenvolvimento reprodutivo. Alguns dados mostram que certos compostos químicos favorecem as translocações cromossomiais e fusões gênicas, possibilitando o surgimento de células clones leucêmicas no período pós-natal **(Greaves, 2003; Pombo-de-Oliveira, 2006)**.

Os mecanismos pelos quais os componentes químicos atuam no desenvolvimento de leucemias ainda são desconhecidos. Entretanto, sabe-se que o acúmulo de mutações em *loci* reguladores das diferentes etapas do processo de diferenciação, proliferação, reparo e apoptose celular podem evoluir para o processo de carcinogênese **(IARC, 1993)**. Além disso, é preciso considerar a importante contribuição dos polimorfismos genéticos na suscetibilidade de desenvolver leucemia na infância **(Sinnett *et al*, 2000)**. Neste sentido, o gene *NAT2 (N-Acetiltransferase 2)*

envolvido na síntese de enzimas de fase II, que atuam na metabolização de agentes cancerígenos, é essencial no processo de acetilação de metabólitos provenientes da oxidação e redução de algumas substâncias medicamentosas **(Zanrosso *et al.,* 2010)**. Sendo assim, o polimorfismo genético do gene *NAT2* resulta em rápida ou lenta acetilação de substâncias potencialmente tóxicas, modificando assim o risco de desenvolvimento de leucemia infantil **(Zanrosso *et al.,* 2010)**.

### 2.5.1. Contribuição dos fatores hereditários no processo leucêmico

A história familiar de câncer é relatada na literatura como um importante fator de risco para estimar a probabilidade pessoal de desenvolver câncer **(Severin, 1999)**. Diante disso, alguns estudos têm analisado a importância do histórico familiar de câncer e o desenvolvimento de vários tipos de neoplasias, entre as quais, estão as leucemias **(Hemminki *et al*, 2001; Infante-Rivarde *et. al*, 2004; Chang *et al*, 2005).**

Um estudo foi realizado na Suécia e na Dinamarca para avaliar a contribuição da história familiar de neoplasias hematológicas e o desenvolvimento de linfomas, observando associação positiva para Linfoma Hodgkin (OR=2,1 IC95%:1,0-4,3) e Linfoma Não-Hodgkin (OR=1,8 IC95%:1,2-2,5) **(Chang *et al*, 2005).** Esse aumento na estimativa de risco foi observado também para outros tipos de neoplasias, como no estudo realizado na Itália entre a história familiar de câncer renal em parentes de primeiro grau e o desenvolvimento de câncer nos rins (OR=5,2 IC95%:2,2-12,2) **(Negri *et al*, 2006)** e o histórico de câncer de mama em parentes de primeiro (RR=2.1 IC95%:2.0-2.2) e segundo grau (RR=1.5 IC95%:1.4-1.6) e a presença de tumores malignos na mama **(Pharoah *et al*, 1997).** Estes achados são importantes para sustentar a hipótese da suscetibilidade genética e o desenvolvimento de diversos tipos de neoplasias no âmbito da história familiar de câncer.

Entretanto, é importante ressaltar que poucos estudos são realizados para avaliar a contribuição da história familiar de câncer no desenvolvimento de neoplasias na infância. O estudo desenvolvido por Hemminki (2001) sugere um aumento no risco de desenvolver leucemia em crianças que apresentam irmãos com algum tipo de tumor. Estes resultados são corroborados por Ripert e colaboradores (2007), que observaram aumento na estimativa de risco na ordem de 60% entre a história familiar de câncer em parentes de primeiro e segundo grau e o desenvolvimento de leucemia em menores de 15 anos.

## 3. Justificativa

O conhecimento produzido a partir de diversos estudos indica que a etiologia das leucemias está associada a um grande número de fatores, sendo estes genéticos e/ou ambientais. Além disso, as variações na suscetibilidade de desenvolver leucemia são observadas entre adultos e crianças devido às diferenças na biologia da doença, como a alta incidência de anormalidades cromossômicas de melhor prognóstico em crianças. Estes fatores são importantes para definir a progressão da doença, determinando assim a sobrevida global dos indivíduos afetados.

Considerando que crianças são particularmente mais vulneráveis aos compostos ambientais, devido à imaturidade do seu metabolismo e às taxas mais elevadas de crescimento e de divisão celular, as investigações epidemiológicas são importantes na contribuição do entendimento da etiologia das leucemias. Além disso, estes estudos podem fornecer informações sobre o prognóstico da doença e a resposta aos agentes quimioterápicos, contribuindo para o desenvolvimento de tratamentos mais eficazes.

Diante disso, é importante considerar que a suscetibilidade do concepto aos diversos fatores de exposição e as evidências de que a origem das leucemias na infância possa ser decorrente da interação entre fatores ambientais e genéticos ainda no período intrauterino. Sendo assim, torna-se oportuno explorar a magnitude de associação entre os aspectos hereditários (como o histórico familiar de câncer) e os fatores ambientais (como o uso de medicamentos), associados ao desenvolvimento de leucemia em crianças menores de dois anos.

Nesse sentido, foi criada uma parceria entre pesquisadores da Escola Nacional de Saúde Pública Sérgio Arouca (**ENSP**) e do Instituto Nacional do Câncer (**INCa**), com o intuito de aprofundar o conhecimento acerca da etiologia das leucemias, contribuindo para a sobrevida destas crianças.

# 4. Objetivos

## 4.1. Objetivo geral

Avaliar a contribuição do histórico familiar de câncer e do uso de medicamentos materno no desenvolvimento de leucemia aguda em crianças menores de dois anos de idade.

## 4.2. Objetivos específicos

**1)** Determinar a magnitude de associação entre os antecedentes de história familiar de câncer de acordo com o grau de parentesco (primeiro ou segundo grau) e o desenvolvimento de leucemias em menores de dois anos de idade.

**2)** Determinar a magnitude de associação entre consumo materno de analgésicos (dipirona, paracetamol e aspirina) no período pré-gestacional, gestacional (1º, 2º e 3º trimestre) e no período de lactação e o desenvolvimento de leucemias agudas em menores de dois anos de idade.

**3)** Determinar a magnitude de associação entre a exposição materna a vitaminas e suplementos minerais no período pré-gestacional, gestacional (1º, 2º e 3º trimestre) e no período de lactação e o desenvolvimento de leucemias agudas em menores de dois anos de idade.

# 5. Metodologia

## 5.1. Desenho e população do estudo

Este trabalho faz parte de um estudo multicêntrico intitulado "Estudo Multi-institucional das Leucemias Infantis: Contribuição dos Marcadores Imuno-moleculares na Distinção de Diferentes Fatores Etiopatogênicos" que tem como foco investigar os mecanismos patogênicos das leucemias agudas da infância no Brasil **(Pombo-de-Oliveira *et al*, 2006; Mansur *et al*, 2009)**.

Trata-se de um estudo caso-controle de base hospitalar, no qual os participantes foram recrutados em 15 unidades hospitalares localizadas em diferentes regiões do Brasil. As crianças foram recrutadas por médicos em hospitais de Belo Horizonte, Brasília, Campinas, Florianópolis, Goiânia, João Pessoa, Recife, Rio de Janeiro, Salvador, Santa Maria e São Paulo, tendo sido obtida uma razão de dois controles para cada caso.

## 5.2. Critérios de inclusão e de exclusão

Para este estudo, os critérios de inclusão para os casos foram crianças com idade inferior a 2 anos, cujo diagnóstico de leucemia foi estabelecido pelos métodos morfológico, imunofenotípico e citogenético-molecular. Os controles foram selecionados entre crianças hospitalizadas nos mesmos hospitais de origem dos casos, tendo a mesma faixa etária e em tratamento médico para outras doenças não malignas **(Emerenciano *et al*, 2006; Mansur *et al*, 2009)**

Diferentes diagnósticos foram incluídos no grupo controle com o intuito de evitar a introdução de viés de seleção, pois determinada patologia poderia estar associada com a exposição de interesse. Entre as patologias diagnosticadas no grupo controle, foram selecionadas aquelas que apresentavam maior gravidade com intuito de

minimizar a introdução de viés de memória. Entre as quais destacam as doenças infecciosas e parasitárias; doenças hematológicas (anemias); doenças respiratórias (asma, bronquite e pneumonia); anomalias congênitas; doenças do aparelho digestivo (gastroenterites) e doenças cardiovasculares (cardiopatias).

Os critérios de exclusão foram crianças com pais adotivos ou biológicos não localizados, portadores de mielodisplasia e síndromes congênitas. Além disso, foram excluídos os casos que apresentaram diagnóstico inconclusivo.

### 5.3. Dados do questionário

O levantamento das informações sobre exposições ambientais potencialmente associadas ao processo de leucemogênese foi realizado mediante o emprego de um instrumento (questionário) padronizado para tal fim. Foram realizadas entrevistas com as mães dos casos e controles para obter informações sobre o perfil socioeconômico da família, sobre a história ocupacional dos pais, bem como os hábitos de vida e de saúde do grupo familiar. Assim, foram obtidas informações sobre a exposição por diversos produtos do período pré-gestacional ao nascimento. Entre os dados coletados constavam informações referentes ao histórico familiar de câncer e o uso de medicamentos estratificado por período pré-concepcional, gestacional e durante a lactação.

As variáveis: peso ao nascer, idade materna no momento do parto, escolaridade, etnia/raça, uso de contraceptivos orais e uso de pesticidas são descritas na literatura como fatores de risco para a leucemia infantil, por isso, foram consideradas como potenciais fatores de confundimento na análise exploratória.

### 5.4. Principais variáveis do estudo

**Sexo da criança:** Masculino ou feminino.

**Cor da criança:** Categorizada em branca, parda, negra, amarela ou indígena.

**Peso ao nascer:** Peso da criança ao nascer em gramas.

**Local de nascimento:** Hospital, cidade e estado brasileiro.

**Idade materna no parto:** Idade materna em anos no momento do parto.

**Escolaridade materna:** Categorizada em analfabeta, ensino fundamental, ensino médio e ensino superior, subdivididos em incompleto ou completo.

**Renda familiar:** Valores da renda mensal em reais do grupo familiar.

**Uso de contraceptivos:** Uso de contraceptivos orais antes e durante a gestação.

**Uso de pesticidas:** Uso de pesticidas, variável dicotômica.

**Informação sobre uso de pesticidas:** Tipo de uso do pesticida (doméstico ou lavoura), marca comercial e duração de uso em meses.

**Histórico familiar de câncer:** Considerado qualquer tipo de câncer na família (primeiro ou segundo grau de parentesco). Além disso, foram obtidos o número de parentes, o subtipo neoplásico e o parentesco com a criança (materno e/ou paterno).

**Uso de analgésicos materno:** No período pré-concepcional, gestacional e durante a lactação, segundo informação materna, variável dicotômica.

**Informação sobre o uso de analgésicos:** Descrição da marca comercial e a duração do uso dos analgésicos.

**Uso de vitaminas materno:** No período pré-concepcional, gestacional e durante a lactação, segundo informação materna, variável dicotômica.

**Informação sobre o uso de vitaminas:** Descrição do nome comercial e a duração do uso de vitamina.

**Uso de suplementos minerais materno:** No período pré-concepcional, gestacional e durante a lactação, segundo informação materna, variável dicotômica.

**Informação sobre o uso de suplementos minerais:** Descrição do nome comercial e a duração do uso de suplementos minerais.

### 5.5. Análise estatística

As diferenças da distribuição de frequência das variáveis selecionadas em casos e controles foram analisadas pelo teste qui-quadrado e pelo teste exato de Fisher (*two-sided*). Além disso, foram determinadas razões de chance (*odds ratio*) ajustadas por variáveis selecionadas (peso ao nascer, idade materna no momento do parto, escolaridade e cor/raça, uso de contraceptivos e exposição a pesticidas), com seus respectivos intervalos de confiança de 95% entre as exposições de interesse e o desenvolvimento de leucemia aguda. Foram realizados modelos multivariados parcimoniosos por meio da regressão logística não condicional pelo pacote estatístico *SPSS 17.0* (*Statistical Package for Social Sciences* - Chicago, IL, 2008).

### 5.6. Aspectos éticos

O estudo multicêntrico de onde se originam os dados foi apreciado e aprovado pelo Comitê de Ética em Pesquisa do Instituto Nacional do Câncer (CEP - INCa, 005/06). A realização desta pesquisa foi aprovada pelo Comitê de Ética da Escola Nacional de Saúde Pública Sérgio Arouca, da Fundação Oswaldo Cruz (CEP-ENSP, 126.638/2012).

Após esclarecimento dos objetivos da investigação, os participantes assinaram um termo de consentimento específico, antes da realização da entrevista, declarando estarem de acordo com os objetivos do estudo.

### 5.7. Apoio Financeiro

Este estudo tem o apoio financeiro do Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (*CNPq*) e da Fundação Swiss Bridge.

## 6. Apresentação dos resultados

Esta tese está estruturada em formato de artigos, composta por três manuscritos:

**1)** Familial history of cancer and leukemia in children under two years old in Brazil.

**2)** Pregnancy, maternal exposure to analgesic medicines and early age leukemia in Brazil.

**3)** Pregnancy, maternal vitamins and mineral supplements intake and leukemia in Brazilian children < 2 years.

### 6.1. Primeiro artigo da tese

**FAMILIAL HISTORY OF CANCER AND LEUKEMIA IN CHILDREN UNDER TWO YEARS OLD IN BRAZIL**

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**Running title:** Familial history cancer and childhood leukemia

**Conflict of Financial Interests**

The authors declare that there are not competing financial conflict of interests.

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**ABSTRACT**

**Objective:** To determine the contribution of familial history of cancer (FHC) in the development of leukemia in children < 2yr.

**Methods**: This is a national hospital-based case-control study with children 0-24 months recruited in 15 Brazilian hospitals from several regions providing oncological care and local general hospitals. Participants FHC antecedents were obtained through face to face interviews with mothers of cases and controls with a standardized questionnaire. Unconditional logistic regression was used to ascertain crude and adjusted (adj) odds ratios (OR), and the respective 95% confidence intervals (C.I.), of acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) after adjustment for selected variables.

**Results:** FHC antecedents were obtained from 178 ALL, 51 AML and 428 controls. FHC in second degree relatives (grandparents, uncles, cousins) showed an adj OR=1.66 (95% C.I. 1.12-2.45) for ALL. Antecedents of 2 or more relatives with cancer showed a statistically significant two fold higher risk of either ALL or AML. Paternal, and joint paternal and maternal antecedents of cancer also showed statistically significant higher adj. OR, respectively 1.80 and 1.89 for ALL, and 2.34 and 3.23 for AML. Hematological malignancies among second degree relatives showed an adj. OR=3.48 (95% C.I. 1.72-7.09) for ALL. According to the anatomic site, antecedents of leukemia/lymphoma among case relatives, comparatively to control ones, showed an OR=2.98 (95% C.I. 1.52-5.82) for ALL, while stomach cancer antecedents revealed an OR=3.55 (95% C.I. 1.02-12.39) for AML.

**Conclusion**: The observed results support the hypothesis that FHC antecedents are associated with leukemogenesis in children < 2yr.

**Keywords:** childhood leukemia; familial history of cancer; ALL; AML; case-control study.

## INTRODUCTION

Childhood cancer can be considered a rare disease, comprising less than 1% of all cancers diagnosed in developing countries [1]. Among the various childhood cancers, leukemia are the most common in childhood, representing about 30% of all diagnoses, being acute lymphoblastic leukemia (ALL) the most frequent and representing 75% of pediatric cases [2]. In Great Britain, all cancer sites incidence estimates under 15 yr. is 144 cases per million [3], with an estimate of leukemia incidence rate of 44.6 per million.

Epidemiological studies have described several possible risk factors for childhood leukemia aiming to enhance understanding of its etiology. Greaves (1999) proposed a model of the possible causal factors, including genetic alterations, environmental factors and acquired modifications [4]. Among the genetic factors, familial history of cancer (FHC) may play an important role in the context of the hypothesis of the etiology of childhood acute leukemia. However, few epidemiological studies have investigated the contribution of hereditary factors and the development of acute leukemia in children [5, 6].

In a recent publication, Schmiegelow and collaborators have reviewed data from an international collaboration program that identified 54 sibships with two or more cases of childhood ALL. The authors proposed that strong genetic risk factors for childhood ALL might be restricted to specific ALL subtypes, and suggested that further epidemiological studies must be taken into account to explore the etiological factors [7].

Considering the availability of epidemiological information obtained from a national study of leukemia in children under 2 yr. in Brazil [8], the aim of this paper was to investigate the contribution of FHC antecedents associated with acute leukemia in early childhood.

# METHODS

## Study population and study design

This study is part of an investigation named "Multi-Institutional Study of Infant Leukemia: Contribution of Immunomolecular Markers in Distinguishing Different Etiopathogenic Factors", which focuses on investigating the pathogenic mechanisms of early age leukemia (EAL) in Brazil. The methodological characteristics of this investigation study have been published elsewhere [8, 9]. In summary, it consisted in a multicentric hospital-based case-control study, performed during 1999-2007, aiming to explore the different risk factors associated to EAL in Brazil. The study included participants recruited from 15 institutions located throughout all regions of the country, except the Amazon, being the participants recruited from hospitals in the cities of Belo Horizonte, Brasília, Campinas, Florianópolis, Goiânia, João Pessoa, Recife, Rio de Janeiro, Salvador, Santa Maria and São Paulo. The studied sample of enrolled participants included a ratio of 2 controls per each EAL recruited case in each participating center.

## Inclusion criteria

Children 0-24 months with an ALL or AML diagnosis established by morphological and immunophenotypical methods, were recruited. Controls were selected among children of the same age range, hospitalized either in the same hospitals or in general hospitals located in the same municipality where the cases came from, and undergoing medical treatment for other non-malignant diseases [10, 11].

In this study, selection bias control was tried to be accomplished by recruiting controls with different diagnosis in order to decrease the probability of including patients with pathologies whose risk factors were potentially associated with the

exposure of interest [8]. Moreover, several life-threatening pathologies (infectious diseases (29%); non-malignant hematological diseases (20%); respiratory diseases (10%); hemangioma (9%); diarrhea (9%); cardiovascular diseases (6%); others (17%)), were selected for the recruitment of controls aiming to also minimize recall bias.

**Exclusion criteria**

Cases and controls exclusion criteria were: having adoptive parents; unknown or unavailable biological parents; myelodysplasia; and congenital syndromes. Patients diagnosed with malignant or benign tumors were also excluded from the control group, given the possible influence of environmental exposures in the etiology of any tumor. Additionally, cases presenting inconclusive diagnosis were also excluded.

**Data Analysis**

Data collection on environmental exposures potentially associated with leukemogenesis was performed through a specifically designed standardized survey. Face to face interviews were carried out with cases and controls mothers in order to obtain information about the socioeconomic background of the family, the parents' occupational history, and the lifestyle and health records of the family, including familial cancer antecedents.

Familial history of cancer was collected for first degree (parents and siblings) and second degree (great grand-parents, grand-parents, uncles, aunts and cousins) relatives of the index child. For each relative with a history of cancer, the mother reported the specific cancer site and the kinship degree with the child. The reported cancer site was then coded using the International Classification of Diseases (ICD-10).

**Statistical analysis**

The statistical significance of the distribution of frequencies of the analyzed variables in cases and controls was determined using the chi-square test and Fisher's exact test (two-sided). Unconditional logistic regression was performed to ascertain the odds ratios (OR) for selected variables (birth weight, maternal education, maternal age, contraceptive use, child skin color, and hormonal and pesticide exposure during pregnancy), and their respective 95% confidence intervals (C.I.), on the association between FHC and EAL. The FHC population-attributable fraction either for ALL or AML was calculated according to the formula [(RR - 1)/RR) * the proportion of cases in the exposed population].

**Ethical Aspects**

This investigation was approved by the Ethics Research Committee of the Brazilian National Cancer Institute (CEP-INCA, 005/06). The mothers of all children (cases and controls) participating in this study signed a consent form agreeing to participate.

**RESULTS**

This study resulted in a sample of 657 participants, including 229 cases (178 ALL and 51 AML) and 428 controls. Familial history of cancer was reported by 313 (47.6%) subjects, of which 123 (39.3%) were EAL cases and 190 (60.7%) controls.

Socio-demographical data revealed participants heterogeneity in the distribution of maternal age at birth, skin color and education **(Table 1)**. In this sense, statistically significant higher proportions of whites, older and more educated mothers were observed among cases than controls.

FHC in first degree relatives was reported by 2 (0.9%) case relatives and 7 (1.6%) control´s. Regarding the presence of a familial history of cancer in second degree relatives, an excess risk estimate was either observed for ALL (adj. OR=1.66 95% C.I.: 1.12-2.45). Comparatively to participants without FHC antecedents, a statistically significant dose-response effect on the risk estimate of EAL was verified according to the amount of relatives with cancer **(Table 2)**. Cases with antecedents of two or more relatives with cancer showed an adj. OR=1.96 (95% C.I. 1.20-3.21) for ALL, and an adj. OR=2.17 (95% C.I. 0.97-4.87) for AML (**Table 2)**. Reported cancer antecedents among great-grandparents showed an adj. OR=2.30 (95% C.I. 1.08-4.93) for AML. Paternal side antecedents of cancer revealed increased risk estimates for ALL (adj. OR=1.80, 95% C.I. 1.04-3.12) and AML (adj. OR=2.34, 95% C.I. 0.96-5.77). When joint paternal and maternal antecedents occurred, the risk estimates were adj. OR=1.89 (95% C.I. 099-3.68) for ALL and adj. OR=3.23 (95% C.I. 1.26-8.29) for AML **(Table 2)**.

The occurrence of antecedents of familial antecedents with any hematopoietic malignancy in second degree relatives showed an adj. OR=3.48 (95% C.I. 1.72-7.09) for AML **(Table 3)**. According to the reported tumor site among participant relatives, familial antecedents of hematologic cancer was more often reported among ALL relatives adj. OR=2.98, 95% C.I. 1.52-5.82, based on 20 case relatives versus 19 controls´ **(Table 4)**. On the other hand, familial antecedents of stomach and bone malignancies were more often reported among AML relatives than control`s, respectively, OR=3.55 95% C.I. 1.02-12.39 (risk estimate based on 4 relative cases with stomach cancer) and OR=9.23, 95% C.I. 1.12-76.3 (risk estimate based on 2 case relatives with bone cancer). The ascertained FHC population-attributable fraction was 20.7% for ALL, and 19.2% for AML.

**Table 1**: Sociodemographic characteristics, leukemia cases and controls, children < 2 yr, Brazil, 1999-2007.

| | | Cases | Controles | |
|---|---|---|---|---|
| | | **n (%)** | **n (%)** | **p- value** |
| **Sex** | | | | |
| | Male | 113 (49.3) | 235 (54.9) | |
| | Female | 116 (50.7) | 193 (45.1) | 0.201 |
| **Skin color** | | | | |
| | White | 132 (57.6) | 175 (40.9) | |
| | No White | 97 (42.4) | 253 (59.1) | 0.001 |
| **Maternal age at birth (yr.)** | | | | |
| | <19 | 11 (4.8) | 76 (17.7) | |
| | 19-24 | 73 (31.9) | 153 (35.7) | |
| | 25-30 | 65 (28.4) | 121 (28.3) | |
| | 31-36 | 57 (24.9) | 53 (12.4) | |
| | >36 | 23 (10.0) | 25 (5.9) | 0.001 |
| **Maternal schooling (years)** | | | | |
| | <9 | 107 (46.7) | 281 (65.7) | |
| | 9 or more | 97 (42.4) | 138 (32.2) | |
| | Unknown | 25 (10.9) | 9 (2.1) | 0.001 |
| **Geographic origin** | | | | |
| | Southeast | 138 (60.3) | 254 (59.3) | |
| | Northeast | 45 (19,7) | 94 (22.0) | |
| | South | 26 (11.3) | 50 (11.7) | |
| | Middle-West | 20 (8.7) | 30 (7.0) | 0.803 |
| **Total** | | **229 (100.0)** | **428 (100.0)** | |

**Table 2:** Antecedents of familial history of cancer among first and second degree relatives, leukemia cases and controls, children < 2 yr., Brazil, 1999-2007.

| Familial History of cancer | Controls (N=428) n (%) | ALL (n=178) n (%) | ALL Crude OR (C.I. 95%) | ALL Adj OR* (C.I. 95%) | AML (n=51) n (%) | AML Crude OR (C.I. 95%) | AML Adj OR* (C.I. 95%) |
|---|---|---|---|---|---|---|---|
| **First degree relatives** | | | | | | | |
| No | 421 (98.4) | 177 (99.4) | 1.00 | 1.00 | 50 (98.0) | 1.00 | 1.00 |
| Yes | 7 (1.6) | 1 (0.6) | 0.34 (0.04-2.78) | 0.35 (0.04-2.91) | 1 (2.0) | 1.20 (0.14-9.98) | 1.00 (0.11-9.24) |
| **Second degree relatives** | | | | | | | |
| No | 246 (57.5) | 85 (47.8) | 1.00 | 1.00 | 23 (45.1) | 1.00 | 1.00 |
| Yes | 182 (42.5) | 93 (52.2) | **1.48 (1.04-2.10)** | **1.66 (1.12-2.45)** | 28 (54.9) | 1.65 (0.92-2.95) | 1.54 (0.80-2.98) |
| **All relatives with cancer** | | | | | | | |
| 0 | 238 (55.6) | 84 (47.2) | 1.00 | 1.00 | 22 (43.1) | 1.00 | 1.00 |
| 1 | 121 (28.3) | 52 (29.2) | 1.22 (0.81-1.83) | 1.35 (0.86-2.11) | 15 (29.4) | 1.34 (0.67-2.68) | 1.21 (0.57-2.59) |
| 2 or more | 69 (16.1) | 42 (23.6) | **1.73 (1.10-2.72)** | **1.96 (1.20-3.21)** | 14 (27.5) | **2.19 (1.07-4.52)** | **2.17 (0.97-4.87)** |
| | | | | **p trend = 0.02** | | | **p trend=0.03** |
| **Grandparents only with cancer** | | | | | | | |
| No | 405 (94.6) | 166 (93.3) | 1.00 | 1.00 | 47 (92.2) | 1.00 | 1.00 |
| Yes | 23 (5.4) | 12 (6.7) | 1.27 (0.62-2.62) | 1.38 (0.64-2.99) | 4 (7.8) | 1.50 (0.50-4.52) | 1.31 (0.40-4.50) |
| **Great-grandparents only with cancer** | | | | | | | |
| No | 387 (90.4) | 156 (87.6) | 1.00 | 1.00 | 41 (80.4) | 1.00 | 1.00 |
| Yes | 41 (9.6) | 22 (12.4) | 1.33 (0.77-2.31) | 1.39 (0.77-2.49) | 10 (19.6) | **2.30 (1.08-4.93)** | 2.15 (0.91-5.10) |
| **Parenthood side** | | | | | | | |
| No antecedents | 238 (55.6) | 84 (47.2) | 1.00 | 1.00 | 22 (43.1) | 1.00 | 1.00 |
| Maternal | 94 (22.0) | 42 (23.6) | 1.43 (0.88-2.31) | 1.27 (0.82-1.97) | 8 (15.7) | 0.92 (0.40-2.14) | 0.95 (0.38-2.35) |
| Paternal | 41 (9.6) | 26 (14.6) | **2.03 (1.13-3.65)** | **1.80 (1.04-3.12)** | 10 (19.6) | **2.64 (1.16-5.98)** | **2.34 (0.96-5.77)** |
| Both | 27 (6.3) | 18 (10.1) | **1.92 (0.97-3.79)** | **1.89 (0.99-3.60)** | 10 (19.6) | **4.00 (1.72-9.34)** | **3.23 (1.26-8.29)** |
| Unspecified | 28 (6.5) | 8 (4.5) | 0.98 (0.42-2.31) | 0.81 (0.36-1.85) | 1 (2.0) | 0.39 (0.05-2.98) | 0.36 (0.04-2.95) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy.

**Table 3:** Antecedents of familial history of solid tumor or hematopoietic malignancies, leukemia cases and controls, children < 2 yr., Brazil, 1999-2007.

| Familial History of Cancer | Controls (N=428) n (%) | ALL (n=178) n (%) | ALL Crude OR (C.I. 95%) | ALL Adj OR* (C.I. 95%) | AML (n=51) n (%) | AML Crude OR (C.I. 95%) | AML Adj OR* (C.I. 95%) |
|---|---|---|---|---|---|---|---|
| **Solid tumors** | | | | | | | |
| First degree relatives | | | | | | | |
| No | 423 (98.9) | 178 (100.0) | - | - | 51 (100.0) | - | - |
| Yes | 5 (1.1) | 0 | | | 0 | | |
| Second degree relatives | | | | | | | |
| No | 226 (55.0) | 103 (57.9) | 1.00 | 1.00 | 23 (45.1) | 1.00 | 1.00 |
| Yes | 203 (45.0) | 75 (42.1) | 1.05 (0.74-1.50) | 1.08 (0.73-1.59) | 28 (54.9) | 1.76 (0.94-3.16) | 1.60 (0.83-3.08) |
| **Hematopoietic malignancies** | | | | | | | |
| First degree relatives | | | | | | | |
| No | 426 (99.1) | 177 (99.4) | 1.00 | 1.00 | 50 (98.0) | 1.00 | 1.00 |
| Yes | 2 (0.9) | 1 (0.6) | 0.80 (0.08-7.74) | 0.66 (0.07-6.56) | 1 (2.0) | 2.83 (0.29-27.75) | 1.39 (0.13-14.97) |
| Second degree relative | | | | | | | |
| No | 408 (91.3) | 159 (89.3) | 1.00 | 1.00 | 50 (98.0) | 1.00 | 1.00 |
| Yes | 20 (8.7) | 19 (10.7) | **3.08 (1.54-6.13)** | **3.48 (1.72-7.09)** | 1 (2.0) | 0.52 (0.07-3.97) | 0.64 (0.08-5.20) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy.

**Table 4:** Reported cancer site among first and second degree relatives, leukemia cases and controls, children < 2 yr., Brazil, 1999-2007.

| Reported cancer site among relatives | Controls (N=428) n (%) | ALL (N=178) n (%) | ALL Crude OR * (C.I. 95%) | ALL Adj OR * (C.I. 95%) | AML (N=51) n (%) | AML Crude OR * (C.I. 95%) | AML Adj OR * (C.I. 95%) |
|---|---|---|---|---|---|---|---|
| **All sites** | 189 (44.2) | 94 (52.8) | **1.41 (1.00-2.01)** | **1.57 (1.06-2.33)** | 29 (56.8) | 1.67 (0.93-2.99) | 1.54 (0.80-2.98) |
| **Leukemia/lymphoma** | 19 (4.4) | 20 (11.2) | **2.72 (1.42-5.24)** | **2.98 (1.52-5.82)** | 2 (3.9) | 0.88 (0.20-3.89) | 0.86 (0.18-4.05) |
| **Stomach** | 17 (4.0) | 10 (5.6) | 1.44 (0.65-3.21) | 1.63 (0.68-3.90) | 4 (7.8) | 2.06 (0.66-6.37) | **3.55 (1.02-12.39)** |
| **Prostate** | 14 (3.3) | 10 (5.6) | 1.76 (0.77-4.04) | 2.13 (0.88-5.13) | 3 (5.9) | 1.84 (0.51-6.66) | 1.91 (0.39-9.26) |
| **Breast** | 38 (8.9) | 20 (11.2) | 1.30 (0.73-2.30) | 1.29 (0.70-2.36) | 8 (15.7) | 1.91 (0.84-4.36) | 1.62 (0.64-4.06) |
| **Oropharynx** | 20 (4.7) | 11 (6.2) | 1.34 (0.63-2.87) | 1.14 (0.51-2.53) | 5 (9.8) | 2.22 (0.79-6.19) | 2.00 (0.62-6.39) |
| **Uterus** | 32 (7.5) | 15 (8.4) | 1.14 (0.60-2.16) | 1.14 (0.58-2.24) | 3 (5.9) | 0.77 (0.23-2.62) | 0.70 (0.19-2.63) |
| **Lung** | 29 (6.8) | 8 (4.5) | 0.65 (0.29-1.45) | 0.58 (0.25-1.35) | 4 (7.8) | 0.56 (0.13-2.43) | 0.26 (0.05-1.38) |
| **Kidney/Bladder** | 6 (1.4) | 6 (3.4) | 2.45 (0.78-7.71) | 2.46 (0.76-8.02) | 1 (2.0) | 1.41 (0.16-11.92) | 1.31 (0.11-15.85) |
| **Colon/Rectum** | 23 (5.4) | 7 (3.9) | 0.72 (0.30-1.71) | 0.61 (0.25-1.51) | 2 (3.9) | 0.72 (0.16-3.14) | 0.58 (0.11-3.01) |
| **Brain** | 4 (0.9) | 4 (2.2) | 2.44 (0.60-9.85) | 2.68 (0.60-11.95) | 0 (0.0) | - | - |
| **Skin** | 7 (1.6) | 4 (2.2) | 1.38 (0.40-4.78) | 1.23 (0.34-4.40) | 2 (3.9) | 2.45 (0.50-12.15) | 2.35 (0.39-14.26) |
| **Liver/biliary tract** | 11 (2.6) | 7 (3.9) | 1.55 (0.59-4.07) | 1.49 (0.54-4.10) | 1 (2.0) | 0.76 (0.10-5.99) | 0.85 (0.10-7.38) |
| **Bones** | 2 (0.5) | 2 (1.1) | 2.42 (0.34-17.32) | 2.25 (0.30-17.03) | 2 (3.9) | **8.69 (1.20-63.10)** | **9.23 (1.12-76.3)** |
| **Oral Cavity/lip** | 5 (1.2) | 6 (3.4) | 2.95 (0.89-9.80) | 3.09 (0.85-11.22) | 0 (0.0) | - | - |
| **Others**** | 9 (2.1) | 7 (3.9) | 1.91 (0.70-5.20) | 1.92 (0.68-5.47) | 2 (3.9) | 1.90 (0.40-9.05) | 2.11 (0.39-11.47) |
| **Unspecified** | 25 (5.8) | 13 (7.3) | 1.27 (0.63-2.54) | 1.35 (0.64-2.81) | 7 (13.7) | 2.56 (0.92-6.27) | 2.83 (0.95-8.27) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy.

**Thyroid, pancreas, head and neck, vagina, retinoblastoma, soft tissue and ear.

## DISCUSSION

Although being leukemia the most common childhood cancer, many aspects of its etiology remain unknown. In this sense, knowledge accomplished from several studies suggests that the causes of leukemia are associated with a large number of genetic and environmental risk factors [12]. In this context, FHC has gained importance over the last decades following increased knowledge on cancer genetics and carcinogenesis. Based on this hypothesis, some studies have analyzed the association between FHC and the development of several neoplasia, such as leukemia [13, 14, 15].

In this sense, the advent of molecular epidemiology has been instrumental in studies involving the mapping of high penetrance genes involved in several cancers showing familial aggregation, such as BRCA1 and BRCA2 involved in ovarian, female and male breast cancer aggregation [16], PMS1 and PMS2 genes associated with the presence of colorectal cancer [17], as well as TP53 tumor suppressor gene whose mutations are frequently detected in several tumors [18].

Considering the contribution of hereditary factors in cancer development, a study to assess the contribution of family history of hematologic malignancies was conducted in Sweden and Denmark, which reported a positive association either for Hodgkin's lymphoma (OR=2.1, 95% C.I. 1.0-4.3) or non-Hodgkin lymphoma (OR=1.8 95% C.I. 1.2-2.5) [15]. Increased risk estimates were also observed for others cancer sites such as kidney and breast cancer [19, 20, 21]. As a whole, all these studies seem to support the hypothesis of genetic susceptibility and development of several types of cancer considering FHC antecedents.

However, few studies have been conducted to evaluate FHC contribution to childhood cancer. Ripert *et al.* (2007) suggested that familial history of solid tumor is

associated with an increased risk of ALL (OR=1.6, 95% C.I. 1.2-2.1), while history of hematopoietic malignancies had a higher risk for AML (OR=4.3, 95% C.I. 1.4-13.0), both observed in first and second degree relatives [6]. An American study conducted with infants also reported a positive but not statically significant association between history of cancer in first degree relatives and the development of AML, OR= 2.2, 95% C.I. 0.6-9.0 [5]. In our study, increased risk estimates on the association between FHC (all sites) and ALL (OR=1.57, 95% C.I. 1.06-2.33) was observed. This finding is in agreement with Ripert et al. (2007), who observed a 60% increase in the estimated risk of childhood leukemia associated to FHC in second degree relatives [6].

Assessing the contribution of family history of solid tumors, Ripert *et al* (2007) observed an increase in the estimated risk for ALL (OR=1.6, 95% C.I. 1.2-2.1), while the family antecedents of hematopoietic malignancies showed an association with AML (OR=4.3, 95% C.I. 1.4-13.0) [6]. In the same direction, a Canadian study found a positive association (OR=2.06, 95% C.I. 1.22-3.49) between the history of hematopoietic diseases in first and second degree relatives and the development of childhood ALL [14]. According to ALL development, our results suggest an increased risk estimate only for family history of hematologic malignancies in second-degree relatives (adj. OR=3.48, 95% C.I. 1.72-7.09).

In our study, an association between FHC in first degree relatives and the development of EAL was not observed. However, Ripert *et al* (2007) reported such association in a population-based study evaluating children under 15 yr [6]. A possible reason for this discrepancy maybe results either from our reduced sample size, just including a small amount of children with reported FHC among first degree relatives (2 case and 7 control relatives), or the age range of our participants parents, relatively young, being cancer incidence low at such age strata.

Regarding the amount of relatives reporting FHC, we observed a quite similar increase in the risk estimate for ALL (OR=1.96, 95% C.I. 1.20-3.21) and AML (OR=2.17, 95% C.I. 0.97-4.87) in families with 2 or more reported relatives with cancer. Moreover, a dose-response effect was observed for both leukemia types according to the amount of affected relatives (p trend=0.02). This association is corroborated by other authors, like Ripert and collaborators (2007), who observed an increase in the risk estimates of childhood leukemia with increasing number of relatives with a history of cancer, OR=1.5, 95% C.I. 1.1-2.0 for families reporting a relative with cancer, and OR=2.3, 95% C.I.1.3-3.8 in families with two or more relatives with cancer) [6]. In our investigation, an association between great-grandparents cancer antecedents and AML was observed. Other epidemiological study exploring the association between grandparents and great-grandparents cancer antecedents, could not obtain statistical significant risk estimates [22].

According to the family history of cancer subtypes, we observed an increased risk estimate between family history of leukemia and the development of ALL (adj. OR=2.98, 95% C.I. 1.52-5.82). Infante-Rivard *et al* (2004) analyzing the importance of family history of cancer subtypes, observed results similar to ours, with a positive association between family history of hematologic malignancies and the development of ALL (OR=2.06, 95% C.I.1.22-3.49) [14]. In analyzing the role of family history of solid tumors, our results suggest that antecedents of either stomach or bones cancer among case relatives, comparatively to control relatives, showed higher risk estimates for AML in children under 2 yr. In other study, increase risk estimates were observed between family history of brain cancer (OR=10.7, 95% C.I. 1.3-86.0), or family history of uterine and ovarian cancer (OR=2.7, 95% C.I. 1.2-5.8) [6].

Our investigation was not a population-based case-control study. Whether our results are comparable to those existing in the general population, the FHC population-attributable fraction ranges around 20% either to ALL or AML.

This investigation presents some limitations. From a study design perspective, as a hospital-based case-control study, selection and information biases introduction cannot be disregarded. However, in order to reduce these potential flaws, cases and controls were recruited in the same cities, if not the same hospitals. In addition, the selected controls were children diagnosed with various diseases, in order to minimize the possible inclusion of selection bias [8]. Additionally, controls were also recruited from children of similar age and presenting severe diseases aiming to restrain a possible recall bias. Other limitation to be mentioned is the lack of information regarding the size of the family and age at diagnosis of cancer among cases and controls relatives, which restrained further analyses exploring patterns of familial aggregation of the disease in the studied sample. Furthermore, one must consider that the obtained information on FHC from the studied children was self-reported by the interviewed mothers, which could have somehow jeopardized data accuracy. However, the reported history of cancer in the family group was similar for cases (46.3%) and controls (55.8%), raising the hypothesis that whether occurring this error, it probably was non-differential among cases and controls.

Regarding the accuracy of information about FHC, some studies have suggested that the introduction of recall bias with differential errors between cases and controls was more frequent according to specificity than sensitivity [23]. In addition, Aitken *et al* (1995) analyzing a family history of cancer and the development of colorectal cancer, observed high negative predictive value and not differential reporting between cases and controls, resulting in high specificity in both groups [24].

On the other hand, this investigation has some strength. So far, to our knowledge, this is the first investigation exploring the association between FHC and childhood leukemia in Latin-America populations. An international collaboration study was performed to address two independent hypotheses genomic and immunological factors by studying families with two or more cases of childhood ALL in a sibships [7]. Data suggested that sibships have been sporadic or occurred randomly, and although genetic susceptibility has not been ruled out, the authors suggested the need of further epidemiological explorative analysis [7].

Secondly, the investigation analyzed such association focusing in a specific age range (children 0-24 months), generally poorly addressed elsewhere according to leukemia risk factors. Standardized procedures to obtain epidemiological data were employed, and participant enrollment was carried following strict guidelines to either restrain possible selection or recall biases. And finally, even considering the particularity of the studied age stratum, the observed results are in quite agreement with the current knowledge about the relationships between FHC and childhood leukemia at older ages. As a whole, all these topics seem to provide a comprehensive bulk of evidence on the validity of the observed results.

In summary, the observed results seem to support the hypothesis that FHC antecedents are associated with leukemogenesis in children under 2 years. Information on FHC should be regularly obtained because it identifies individuals in family groups with higher amount of reported cancer cases, comparatively to the general population. Therefore, it enables a differentiated monitoring of such high risk groups, including more frequent screening and periodic clinical evaluation, thus enabling improvement in cancer control policies organization, including those towards childhood leukemia.

**APPENDIX**. List of pediatricians from *Brazilian Collaborative Study Group of Infant Acute Leukemia* who have contributed as co-authors to the study.

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## 6.2. Segundo artigo da tese

# PREGNANCY, MATERNAL EXPOSURE TO ANALGESIC MEDICINES AND EARLY AGE LEUKEMIA IN BRAZIL

**Running title:** Pregnancy, analgesic use and childhood leukemia

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## ABSTRACT

**Background:** Childhood leukemia etiology remains largely unexplored, mainly in relation to the interaction of genetic and environmental risk factors.

**Methods**: This is a national hospital-based case-control study carried out among children 0-23 months old recruited in cancer and general hospitals from several regions in Brazil. Maternal use of medicines (analgesics included) during pregnancy was obtained through face-to-face interviews with mothers of 231 leukemia cases and 411 controls. Unconditional logistic regression was used to calculate crude and adjusted (adj) odds ratios (OR), and their 95% confidence intervals, on the association between early age leukemia (EAL) and analgesics use during pregnancy.

**Results:** Reported use of acetaminophen during the first trimester of pregnancy showed an OR=0.39 (95% C.I. 0.17-0.93) for acute lymphocytic leukemia (ALL) and an OR=0.37 (95% C.I. 0.16-0.88) for the use in second trimester of pregnancy. Regarding acute myeloid leukemia (AML), an OR=0.11 (95% C.I. 0.02-0.97) for use in the second trimester was found. For ALL cases, exclusive use of dipyrone during pre-conception showed an OR=1.63 (95% C.I. 1.06-2.53) and an OR=2.00 (95% C.I. 1.18-3.39) was found for use of dipyrone during lactation.

**Conclusion**: These results suggest that acetaminophen use during pregnancy may have a protective effect in EAL development in the offspring, while dipyrone use may act as a risk factor for such outcome.


**Keywords:** childhood leukemia; analgesic; pregnancy; case-control study.

## INTRODUCTION

Leukemia is the most common form of childhood cancer, accounting for approximately 30-40% of all diagnosed cases of cancer **(Stiller, 2004)**. Although the causes of leukemia remain unknown, it is unlikely that the leukemic transformation may be the result of a single event, but rather the accumulation of a multiple step process involving interactions between environmental and genetic factors **(Greaves, 2003; Belson, 2007; Dickinson, 2005; Wiemels, 2012).** Maternal use of medicines before or during pregnancy remains a public health problem worldwide, which can lead to the development of congenital malformations, low birth weight and delayed physical development of the child **(Khera, 1987; Broekhuizen *et al.* 1992, D'Apólito, 1998)**. Additionally, recent studies support the hypothesis on the intrauterine etiology of leukemia, stressing the problems caused by fetal exposure to toxic agents in early gestation **(Ognjanovic *et al*, 2011; Wiemels, 2012).**

A survey conducted in Southern Brazil observed that analgesics represented 44.5% of prescription drugs used during pregnancy, being dipyrone the most consumed medicine in the 3rd trimester (41.8%), followed by aspirin and acetaminophen (20.9% either) **(Fonseca *et al*, 2002).** A multicenter study in Brazil reported an increase in the risk estimates for development of leukemia under the age of 2 years (OR=1.45; 95% C.I. 1.02-2.06) associated with the use of dipyrone during pregnancy **(Pombo-de-Oliveira *et al.* 2006)**. Moreover, maternal use of dipyrone during pregnancy was also associated with the development of Wilms' tumor in Brazil (OR=10.9; 95% C.I. 2,4-50,0) **(Sharpe & Franco, 1996).** However, few studies with controversial results have evaluated the effects of other analgesics (such as acetaminophen and aspirin) on the development of childhood leukemia **(Shaw *et al*, 2004; Schuz et al, 2007; Ognjanovic *et al*, 2011).**

The aim of this study was to explore the association between analgesic use during pre-conception, pregnancy and lactation, and the development of acute leukemia in children younger than 2 years of age.

## METHODS

### Study population and design

This study is part of the "Multi-Institutional Study of Infant Leukemia: Contribution of Immunomolecular Markers in Distinguishing Different Etiopathogenic Factors", which focuses on the pathogenic mechanisms of early-age (0-23 months) leukemia (EAL) in Brazil. The methodological characteristics of this multicentric study have been previously published **(Pombo-de-Oliveira *et al*., 2006; Mansur *et al*., 2009).** Briefly, it is a multicentric hospital-based case-control study performed between 1999 and 2007 with the aim of exploring the different risk factors associated with EAL in Brazil.

### Inclusion criteria

In the present study, the inclusion criteria were having been diagnosed of acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML) by morphological and immunophenotypical methods before the age of 2 years. Cases were recruited from 15 institutions located in cities from all regions of the country, excepting the North region, i.e. Belo Horizonte, Brasília, Campinas, Florianópolis, Goiânia, João Pessoa, Recife, Rio de Janeiro, Salvador, Santa Maria, and São Paulo. Controls were selected among children in the same age range, which were hospitalized in the same hospitals as cases or in general hospitals located in the same municipality where cases came from, and undergoing medical treatment for other non-malignant diseases **(Pombo-de-Oliveira *et al*., 2009).**

To avoid (or to minimize) selection bias, controls with several pathologies were enrolled. Such procedure was carried out aiming to reduce the probability of including patients with illnesses with risk factors potentially associated with the exposure of interest**.** To minimize the introduction of recall bias, life-threatening pathologies were chosen for selection of controls, including infectious diseases (29%), non-malignant hematological diseases (20%), respiratory diseases (10%), hemangioma (9%), diarrhea (9%), cardiovascular diseases (6%), and others (17%). The final study sample had a ratio of 2 controls per each EAL recruited case.

**Exclusion criteria**

Cases and controls were excluded if they had unknown or unavailable biological parents, myeloid dysplasia, or congenital syndromes. Patients diagnosed with malignant or benign tumors were also excluded, given the potential influence of environmental exposures in the etiology of any tumor. Additionally, cases presenting inconclusive diagnosis were also excluded.

**Data collection**

Information on environmental exposures potentially associated with leukemogenesis was gathered through a specifically-designed and standardized questionnaire. Face-to-face interviews were carried out with mothers of cases and controls in order to obtain information about the socioeconomic background of the family, parents' occupational history, and lifestyle and family health history, including the use of drugs and the respective time window of exposure to analgesics (3 months before pregnancy, pregnancy trimesters, and during the 3 months after birth). Data

regarding trademarks of drugs, including analgesics, were reported by mothers of cases and controls for further analysis of specific medicines use.

**Statistical analysis**

The distribution of frequencies of the variables related to cases and controls was determined, and subsequently compared by using the chi-square test and Fisher's exact test (two-sided). Unconditional logistic regression was performed to ascertain the magnitude of association between the use of specific analgesic drugs and EAL. Crude and adjusted odds ratios (OR) were obtained after controlling for selected variables (birth weight, maternal education, maternal age, contraceptive use, child skin color, and hormonal use and pesticide exposure during pregnancy), as well as their respective 95% confidence intervals (C.I.). The association between *MLL* rearrangements and exposure to analgesic was also explored.

**Ethical Aspects**

This investigation was approved by the Ethics Research Committee of the Brazilian National Cancer Institute (CEP-INCA, 005/06) and the Ethical Committee of the National Public Health School, Oswaldo Cruz Foundation (CEP-ENSP, 126-638/2012). The mothers of all children (cases and controls) participating in this study signed an informed consent form agreeing to participate.

**RESULTS**

The present study recruited a sample of 642 participants, including 231 cases (176 ALL and 55 AML) and 411 controls with available information about maternal analgesic use during pregnancy. Maternal use of analgesics during the studied exposure

windows was reported by 57.9 % of ALL's mothers, 58.2% of AML's mothers, and 55.0% of controls' mothers. The analgesic drugs most reported by participating mothers were dipyrone (73.5%), acetaminophen (35.5%), and aspirin (10.9%) during pregnancy.

Socio-demographic characteristics of participants revealed differences in the distribution of maternal age at birth, skin color, and education between cases and controls. Thus, cases were more likely to be white than controls (53.2% and 38.0%, respectively, $p<0.01$), and their mothers were older and had higher schooling than controls **(Table 1).**

Regarding ALL, a protective effect following exclusive use of acetaminophen during pregnancy was observed, with an adjusted (adj) OR=0.39 (95% C.I. 0.17-0.93) for reported maternal use during the first trimester, and an adj. OR=0.37 (95% C.I. 0.16-0.88) during the second trimester of pregnancy **(Table 2).**

Higher ALL risk estimates were observed as associated with the reported use of dypirone during pre-conception (adj. OR=1.63, 95% C.I. 1.06-2.53) and breastfeeding (adj. OR=2.00, 95% C.I. 1.18-3.39). In relation to EAL development, either the protective effect of acetaminophen or the risk for dypirone use during pregnancy was observed in the first year of life **(Table 3).** No association was observed between aspirin use and EAL in the offspring (data not shown).

The magnitude of the association between maternal exclusive use of dypirone during pregnancy and the presence of *MLL* gene rearrangements in the offspring was adj. OR=2.89 (95% C.I. 1.25-6.68). Risk estimates found in all the studied time windows of exposure showed statistically significant ORs equal or higher than 2.50 **(Table 4).**

**Table 1**: Socio-demographic characteristics, leukemia cases and controls, children < 2 yr, Brazil, 1999-2007.

| | Cases | Controles | |
|---|---|---|---|
| | **n (%)** | **n (%)** | **p- value** |
| **Sex** | | | |
| Male | 124 (53.7) | 222 (54.0) | |
| Female | 107 (46.3) | 189 (46.0) | 0.93 |
| **Skin color** | | | |
| White | 123 (53.2) | 156 (38.0) | |
| No White | 108 (46.8) | 255 (62.0) | 0.01 |
| **Maternal age at birth (yr.)** | | | |
| <19 | 13 (5.6) | 76 (18.0) | |
| 19-24 | 80 (34.6) | 153 (38.7) | |
| 25-30 | 66 (28.6) | 121 (24.6) | |
| 31-36 | 52 (22.5) | 53 (13.1) | |
| >36 | 20 (8.7) | 25 (5.6) | 0.001 |
| **Maternal schooling (years)** | | | |
| <9 | 125 (54.1) | 281 (68.4) | |
| 9 or more | 106 (45.9) | 130 (31.6) | 0.001 |
| **Geographic origin** | | | |
| Southeast | 134 (58.0) | 226 (55.0) | |
| Northeast | 52 (22.5) | 101 (24.6) | |
| South | 28 (12.1) | 53 (12.9) | |
| Middle-West | 17 (7.4) | 31 (7.5) | 0.90 |
| **Total** | **231 (100.0)** | **411 (100.0)** | |

**Table 2:** Maternal exposure to analgesic during pregnancy, leukemia cases controls, children < 2 yr., Brazil, 1999-2007.

| Analgesic | Controls N (%) | ALL N (%) | ALL Crude OR (95% CI) | ALL Adj. OR* (95% CI) | AML N (%) | AML Crude OR (95% CI) | AML Adj. OR * (95% CI) |
|---|---|---|---|---|---|---|---|
| **Reported** | | | | | | | |
| No | 185 (45.0) | 74 (42.0) | 1.00 | 1.00 | 23 (41.8) | 1.00 | 1.00 |
| Acetaminophen only | 60 (14.6) | 17 (9.7) | 0.71 (0.39-1.29) | 0.53 (0.28-1.02) | 6 (10.9) | 0.80 (0.31-2.07) | 0.48 (0.16-1.43) |
| Dypirone only | 136 (33.1) | 70 (39.8) | 1.29 (0.87-1.91) | 1.41 (0.92-2.16) | 20 (36.4) | 1.18 (0.62-2.24) | 1.13 (0.55-2.35) |
| Both | 30 (7.3) | 15 (8.5) | 1.25 (0.64-2.46) | 0.86 (0.41-1.83) | 6 (10.9) | 1.61 (0.61-4.28) | 1.35 (0.46-3.97) |
| **Pre-conception **** | | | | | | | |
| No | 261 (63.5) | 98 (55.7) | 1.00 | 1.00 | 23 (41.8) | 1.00 | 1.00 |
| Acetaminophen only | 27 (6.6) | 12 (6.8) | 1.18 (0.58-2.43) | 0.80 (0.37-1.73) | 6 (10.9) | 0.80 (0.31-2.07) | 0.48 (0.16-1.43) |
| Dypirone only | 100 (24.3) | 56 (31.8) | **1.49 (1.00-2.23)** | **1.63 (1.06-2.53)** | 20 (36.4) | 1.18 (0.62-2.24) | 1.13 (0.55-2.35) |
| Both | 23 (5.6) | 10 (5.7) | 1.16 (0.53-2.52) | 0.82 (0.35-1.94) | 6 (10.9) | 1.61 (0.61-4.28) | 1.35 (0.46-3.97) |
| **1st Trimester** | | | | | | | |
| No | 269 (65.5) | 118 (67.0) | 1.00 | 1.00 | 26 (47.3) | 1.00 | 1.00 |
| Acetaminophen only | 44 (10.7) | 11 (6.3) | **0.45 (0.20-1.00)** | **0.38 (0.16-0.87)** | 3 (5.5) | 0.54 (0.16-1.87) | 0.44 (0.12-1.63) |
| Dypirone only | 78 (19.0) | 39 (22.2) | 1.24 (0.85-1.81) | 1.36 (0.90-2.05) | 22 (40.0) | 1.18 (0.64-2.16) | 1.24 (0.62-2.47) |
| Both | 20 (4.9) | 8 (4.5) | 1.27 (0.59-2.76) | 0.94 (0.40-2.23) | 4 (7.3) | 1.48 (0.47-4.65) | 1.31 (0.37-4.59) |
| **2nd Trimester** | | | | | | | |
| No | 267 (65.0) | 118 (67.0) | 1.00 | 1.00 | 27 (49.1) | 1.00 | 1.00 |
| Acetaminophen only | 45 (10.9) | 13 (7.4) | **0.47 (0.21-1.03)** | **0.34 (0.15-0.80)** | 2 (3.6) | 0.36 (0.08-1.56) | 0.20 (0.04-1.08) |
| Dypirone only | 80 (19.5) | 38 (21.6) | 1.24 (0.85-1.81) | 1.35 (0.89-2.04) | 21 (38.2) | 1.10 (0.60-2.02) | 1.12 (0.56-2.23) |
| Both | 19 (4.6) | 7 (4.0) | 1.33 (0.63-2.81) | 0.93 (0.40-2.13) | 5 (9.1) | 1.71 (0.60-4.89) | 1.40 (0.44-4.49) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **3rd Trimester** | | | | | | | |
| No | 274 (66.7) | 121 (68.8) | 1.00 | 1.00 | 40 (72.7) | 1.00 | 1.00 |
| Acetaminophen only | 45 (10.9) | 15 (8.5) | 0.76 (0.40-1.41) | 0.56 (0.28-1.11) | 4 (7.3) | 0.61 (0.21-1.78) | 0.50 (0.16-1.62) |
| Dypirone only | 72 (17.5) | 32 (18.2) | 1.00 (0.63-1.61) | 1.20 (0.71-1.99) | 8 (14.5) | 0.76 (0.34-1.70) | 0.99 (0.40-2.46) |
| Both | 20 (4.9) | 8 (4.5) | 0.90 (0.39-2.11) | 0.81 (0.33-2.02) | 3 (5.5) | 1.03 (0.29-3.62) | 1.02 (0.26-4.03) |
| **Breastfeeding*** ** | | | | | | | |
| No | 314 (76.4) | 124 (70.5) | 1.00 | 1.00 | 44 (80.0) | 1.00 | 1.00 |
| Acetaminophen only | 27 (6.6) | 10 (5.7) | 0.94 (0.44-1.99) | 0.67 (0.30-1.54) | 3 (5.5) | 0.79 (0.23-2.72) | 0.61 (0.16-2.27) |
| Dypirone only | 56 (13.6) | 36 (20.5) | **1.63 (1.03-2.60)** | **2.00 (1.18-3.39)** | 6 (10.9) | 0.77 (0.31-1.88) | 0.90 (0.32-2.55) |
| Both | 14 (3.4) | 6 (3.4) | 1.08 (0.40-2.89) | 1.04 (0.36-2.99) | 2 (3.6) | 1.02 (0.22-4.64) | 0.93 (0.17-5.21) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

** 3 months before birth *** 3 months after birth.

**Table 3:** Maternal exposure to analgesic during pregnancy, ALL cases and controls, children < 2 yr., Brazil, 1999-2007.

| | 0-11 months | | | | 12-23 months | | | |
|---|---|---|---|---|---|---|---|---|
| **Analgesic** | **Controls N (%)** | **ALL N (%)** | **Crude OR (95% CI)** | **Adj. OR* (95% CI)** | **Controls N (%)** | **ALL N (%)** | **Crude OR (95% CI)** | **Adj. OR * (95% CI)** |
| **Reported** | | | | | | | | |
| No | 122 (45.4) | 25 (29.8) | 1.00 | 1.00 | 63 (44.4) | 49 (53.3) | 1.00 | 1.00 |
| Acetaminophen | 40 (14.9) | 10 (11.9) | 1.22 (0.54-2.76) | 0.69 (0.27-1.74) | 20 (14.1) | 7 (7.6) | 0.45 (0.18-1.15) | 0.41 (0.15-1.15) |
| Dypirone | 86 (32.0) | 41 (48.8) | **2.33 (1.32-4.11)** | **2.23 (1.19-4.20)** | 50 (35.2) | 29 (31.5) | 0.75 (0.41-1.35) | 1.02 (0.52-2.00) |
| Both | 21 (7.8) | 8 (9.5) | 1.86 (0.74-4.67) | 0.87 (0.29-2.65) | 9 (6.3) | 7 (7.6) | 1.00 (0.35-2.87) | 0.98 (0.30-3.19) |
| **Pre-conception **** | | | | | | | | |
| No | 180 (66.9) | 40 (47.6) | 1.00 | 1.00 | 81 (57.0) | 58 (63.0) | 1.00 | 1.00 |
| Acetaminophen | 16 (5.9) | 5 (6.0) | 1.40 (0.49-4.06) | 0.65 (0.19-2.23) | 11 (7.7) | 7 (7.6) | 0.89 (0.32-2.43) | 0.78 (0.25-2.44) |
| Dypirone | 58 (21.6) | 35 (41.7) | **2.72 (1.58-4.67)** | **2.90 (1.58-5.35)** | 42 (29.6) | 21 (22.8) | 0.70 (0.37-1.30) | 0.88 (0.44-1.76) |
| Both | 15 (5.6) | 4 (4.8) | 1.20 (0.38-3.81) | 0.49 (0.12-2.02) | 8 (5.6) | 6 (6.5) | 1.05 (0.35-3.19) | 0.93 (0.27-3.20) |
| **1st Trimester** | | | | | | | | |
| No | 134 (49.8) | 32 (38.1) | 1.00 | 1.00 | 68 (47.9) | 51 (55.4) | 1.00 | 1.00 |
| Acetaminophen | 28 (10.4) | 3 (3.6) | 0.45 (0.13-1.57) | **0.25 (0.10-0.95)** | 15 (10.6) | 5 (5.4) | 0.44 (0.15-1.30) | 0.50 (0.15-1.66) |
| Dypirone | 92 (34.2) | 44 (52.4) | **2.00 (1.18-3.39)** | **1.99 (1.09-3.60)** | 53 (37.3) | 30 (32.6) | 0.76 (0.42-1.34) | 0.98 (0.51-1.89) |
| Both | 15 (5.6) | 5 (6.0) | 1.40 (0.47-4.12) | 0.53 (0.13-2.15) | 6 (4.2) | 6 (6.5) | 1.33 (0.41-4.38) | 2.08 (0.55-7.94) |
| **2nd Trimester** | | | | | | | | |
| No | 134 (49.8) | 32 (38.1) | 1.00 | 1.00 | 69 (48.6) | 51 (55.4) | 1.00 | 1.00 |
| Acetaminophen | 28 (10.4) | 3 (3.6) | 0.45 (0.13-1.57) | 0.29 (0.07-1.13) | 14 (9.9) | 5 (5.4) | 0.48 (0.16-1.43) | 0.40 (0.12-1.34) |
| Dypirone | 92 (34.2) | 43 (51.2) | **1.96 (1.15-3.32)** | **2.01 (1.11-3.64)** | 52 (36.6) | 30 (32.6) | 0.78 (0.44-1.39) | 1.02 (0.53-1.96) |
| Both | 15 (5.6) | 6 (7.1) | 1.67 (0.60-4.66) | 0.65 (0.18-2.40) | 7 (4.9) | 6 (6.5) | 1.16 (0.37-3.66) | 1.41 (0.38-5.20) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **3rd Trimester** | | | | | | | | |
| No | 175 (65.1) | 49 (58.3) | 1.00 | 1.00 | 99 (69.7) | 72 (78.3) | 1.00 | 1.00 |
| Acetaminophen | 32 (11.9) | 8 (9.5) | 0.89 (0.39-2.06) | 0.50 (0.19-1.32) | 13 (9.2) | 7 (7.6) | 0.74 (0.28-1.95) | 0.75 (0.26-2.21) |
| Dypirone | 48 (17.8) | 23 (27.4) | 1.71 (0.95-3.08) | **1.95 (1.01-3.77)** | 24 (16.9) | 9 (9.8) | 0.52 (0.23-1.18) | 0.65 (0.25-1.65) |
| Both | 14 (5.2) | 4 (4.8) | 1.02 (0.32-3.24) | 0.65 (0.17-2.54) | 6 (4.2) | 4 (4.3) | 0.92 (0.25-3.37) | 1.20 (0.28-5.03) |
| **Breastfeeding*** ** | | | | | | | | |
| No | 206 (76.6) | 54 (64.3) | 1.00 | 1.00 | 108 (76.1) | 70 (76.1) | 1.00 | 1.00 |
| Acetaminophen | 17 (6.3) | 4 (4.8) | 0.90 (0.29-2.78) | 0.51 (0.14-1.82) | 10 (7.0) | 6 (6.5) | 0.93 (0.32-2.66) | 0.81 (0.24-2.75) |
| Dypirone | 37 (13.8) | 24 (28.6) | **2.47 (1.36-4.49)** | **2.92 (1.46-5.86)** | 19 (13.4) | 12 (13.0) | 0.97 (0.44-2.13) | 1.27 (0.51-3.13) |
| Both | 9 (3.3) | 2 (2.4) | 0.85 (0.18-4.04) | 0.52 (0.09-3.14) | 5 (3.5) | 4 (4.3) | 1.23 (0.32-4.76) | 1.94 (0.43-8.83) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

** 3 months before birth *** 3 months after birth.

**Table 4:** Maternal exposure to analgesic during pregnancy, *MLLr* cases and controls, children < 1 yr., Brazil, 1999-2007.

| **Analgesic** | Controls N (%) | MLLr n (%) | Crude OR (95% CI) | Adj. OR (95%) |
|---|---|---|---|---|
| **Reported** | | | | |
| No | 122 (45.4) | 10 (23.3) | 1.00 | 1.00 |
| Acetaminophen | 40 (14.9) | 6 (14.0) | 1.83 (0.63-5.35) | 1.55 (0.49-4.94) |
| Dypirone | 86 (32.0) | 21 (48.8) | **2.98 (1.34-6.64)** | **2.89 (1.25-6.68)** |
| Both | 21 (7.8) | 6 (14.0) | **3.49 (1.15-10.61)** | 2.49 (0.75-8.25) |
| **Pre-conception **** | | | | |
| No | 180 (66.9) | 19 (44.2) | 1.00 | 1.00 |
| Acetaminophen | 16 (5.9) | 3 (7.0) | 1.78 (0.47-6.65) | 1.07 (0.25-4.59) |
| Dypirone | 58 (21.6) | 18 (41.9) | **2.94 (1.45-5.98)** | **2.96 (1.40-6.28)** |
| Both | 15 (5.6) | 3 (7.0) | 1.89 (0.50-7.14) | 1.22 (0.59-5.15) |
| **1st Trimester** | | | | |
| No | 134 (49.8) | 14 (32.6) | 1.00 | 1.00 |
| Acetaminophen | 28 (10.4) | 2 (4.7) | 0.68 (0.15-3.18) | 0.45 (0.09-2.36) |
| Dypirone | 92 (34.2) | 26 (60.5) | **2.71 (1.34-5.46)** | **2.60 (1.24-5.43)** |
| Both | 15 (5.6) | 1 (2.3) | 0.64 (0.08-5.20) | 0.35 (0.04-3.45) |
| **2nd Trimester** | | | | |
| No | 134 (49.8) | 14 (32.6) | 1.00 | 1.00 |
| Acetaminophen | 28 (10.4) | 2 (4.7) | 0.68 (0.15-3.18) | 0.58 (0.11-2.93) |
| Dypirone | 92 (34.2) | 24 (55.8) | **2.50 (1.23-5.08)** | **2.50 (1.18-5.27)** |
| Both | 15 (5.6) | 3 (7.0) | 1.91 (0.49-7.43) | 1.18 (0.27-5.16) |
| **3rd Trimester** | | | | |
| No | 175 (65.1) | 21 (48.8) | 1.00 | 1.00 |
| Acetaminophen | 32 (11.9) | 5 (11.6) | 1.30 (0.46-3.70) | 1.21 (0.39-3.76) |
| Dypirone | 48 (17.8) | 15 (34.9) | **2.60 (1.25-5.43)** | **2.78 (1.25-6.18)** |
| Both | 14 (5.2) | 2 (4.7) | 1.19 (0.25-5.60) | 0.89 (0.17-4.72) |
| **Breastfeeding***** | | | | |
| No | 206 (76.6) | 24 (55.8) | 1.00 | 1.00 |
| Acetaminophen | 17 (6.3) | 2 (4.7) | 1.01 (0.22-4.64) | 0.82 (0.16-4.13) |
| Dypirone | 37 (13.8) | 16 (37.2) | **3.71 (1.80-7.65)** | **3.87 (1.72-8.68)** |
| Both | 9 (3.3) | 1 (2.3) | 0.95 (0.12-7.86) | 0.57 (0.06-5.76) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

** 3 months before birth *** 3 months after birth.

## DISCUSSION

Analgesics are one of the most prescribed classes of drugs consumed during pregnancy in Brazil **(Fonseca *et al*, 2002).** In other countries, these drugs are also easily purchased over-the-counter, being misused most of the time **(Glover *et al*, 2003).** Most analgesics belong to a class of drugs named *Non-Stereoidal Antiinflamatory Drugs* (NSAIDs), which inhibit the cyclooxygenase (COXs) enzymes, thus controlling the inflammation, analgesia and hyperthermia **(Burdan *et al*, 2006).** In the present investigation, the analgesics that were reported to be used more often by mothers of cases and controls during the studied time exposure windows were dipyrone, acetaminophen and aspirin.

The use of COX inhibitors drugs during pregnancy has been reported to be associated with prematurity, miscarriage and congenital malformations, such as cardiac and diaphragmatic alterations **(Ostensen & Skomsvoll, 2004).** Unlike dipyrone and aspirin, acetaminophen is a selective COX-3 inhibitor, not showing an anti-inflammatory effect. Nevertheless, some authors have suggested that acetaminophen and NSAIDs share biochemical pathways such as suppression of NF-kappaB activity, which can decrease transcription of growth factors, chemokines and proteases **(Altino & Korkmarz, 2004).** Hence, both drugs could be involved in the cancer-growth-stimulating inflammatory stimuli, rather than in the direct cellular toxicity **(Altino & Korkmarz, 2004).**

During the last decades, acetaminophen has been evaluated as the safest antipyretic and analgesic drug to be consumed during pregnancy **(Graham & Scott, 2005).** Depending on the structure and the type of chemical bond, different pathological effects may occur in the developing fetus, such as mutagenesis, carcinogenesis and teratogenesis **(Oshima-Franco & Franco, 2003).** However, it is important to consider that biotransformation is a paramount step in the process of elimination and toxicity

reduction of chemical substances. Furthermore, biotransformation may also be responsible for the formation of intermediate reactive metabolites, which bind to body macromolecules such as DNA **(Oshima-Franco & Franco, 2003).**

Therefore, the discussion about the use of medications during pregnancy is relevant because the mechanisms leading to congenital diseases are directly associated with the reproductive toxicity of chemicals **(Ostensen & Skomsvoll, 2004).** Accordingly, it is a general rule that any medicine consumed during pregnancy should be ingested in low therapeutic doses, thus reducing the potential risk of adverse effects in the developing fetus **(Ostensen & Skomsvoll, 2004).**

Despite widespread use of acetaminophen during pregnancy, information about the effects of toxicity due to overdose are extremely rare. A cohort of 48 pregnant women exposed to acetaminophen overdoses was monitored and teratogenic effects were not observed **(McElhatton *et al*., 1990).** Similar results were observed in other studies, and there was a lack of evidence of teratogenic effects following paracetamol exposure in the second and third trimesters of pregnancy **(Rayburn *et al.,* 1984, Riggs *et al.,* 1989).** The results of the current investigation suggest a protective effect of maternal use of acetaminophen during pregnancy on ALL development in the offspring, especially among those mothers who used this analgesic during early pregnancy (first and second trimester).

A study in the USA found a borderline protective effect between the use of NSAIDs, except aspirin (OR=0.60, 95% C.I. 0.37-0.97) and acetaminophen (OR=0.66, 95% C.I. 0.43-1.01) on the development of AML in children under 1 year of age **(Ognjanovic *et al*, 2011).** However, the protective effect following aspirin use was not statistically significant (OR=0.55, 95% C.I. 0.24-1.26) **(Ognjanovic *et al*, 2011).** The same study showed that acetaminophen was also consumed before and after knowledge of pregnancy, respectively, 63 and 69%.

Dipyrone use was banned in several countries in late 70's, including the USA, Australia, Japan and most European countries **(Banned Products, 1997).** However, in some other countries like Brazil, Mexico, India, Russia and Turkey, dipyrone is still commercialized, being one of the most consumed analgesics in Brazil. Among the serious side effects caused by the use of dipyrone, hemolytic anemia and bone marrow aplasia have been reported **(Anon, 1986; Kulkarni *et al,* 1985).**

The bone marrow failure may lead to agranulocytosis, a rare disease presenting a death fatality rate of around 6-10%, which resulted in the prohibition of dipyrone commercialization in many countries **(Banned Products, 1997).** Importantly, studies describing the incidence of agranulocytosis due to consumption of dipyrone show geographical variability **(Hamerschlak & Cavalcanti, 2005).** This geographic variability may be partly explained by differences in patterns of use, dose, duration of treatment and concomitant use of other drugs **(Ibanez *et al*, 2005).** Results of the current investigation suggest a positive association between maternal use of dipyrone during preconception, the first trimester of pregnancy and breastfeeding, and the development of ALL in the offspring before the age of 1 year. However, when exploring the effect of the combined use of dipyrone and acetaminophen, such increased risk no longer persisted, suggesting the existence of an additive effect due to intake of the two analgesics is negligible. The observation of an over 3-fold higher risk for the occurrence of *MLL* gene rearrangements in children 0-11 months associated with the maternal exclusive use of dypirone during pregnancy seems to be a relevant contribution supporting the potential toxic effect of dypirone leading to leukemogenesis at a such early age.

Regarding aspirin, its use is not recommended during pregnancy, particularly in the third trimester of pregnancy **(Werler *et al,* 1989; Kozer *et al*, 2002).** In the current study, aspirin use during pregnancy was the analgesic use less reported by case and

control mothers, and statistically significant EAL risk estimates were not observed (data not shown).

Other studies did not report an association between analgesic use during pregnancy and the development of leukemia in children under of age 15 years **(Wen *et al,* 2002; Shaw *et al,* 2004; Schuz *et al*, 2007).** Shaw and colleagues (2004) observed a positive association between the use of any medication during pregnancy and the development of leukemia in children under 15 years (OR=1.3, 95% C.I. 1.0-1.6). However, there was no statistically significant association between the consumption of analgesics during pregnancy and the development of childhood leukemia (OR=1.9, 95% C.I. 0.9-4.1). Wen and colleagues (2002) also found no significant association between the use of pain relievers during pregnancy and the development of leukemia in children under 15 years (OR = 1.1, 95% CI 0.5-2.1). A limitation of these studies was to evaluate drug groups, without detailing the specific medication consumed by pregnant women.

Our investigation presents some limitations. As a hospital-based case-control study, selection and information biases introduction cannot be disregarded. However, in order to reduce these potential flaws, cases and controls were recruited in the same cities, or in the same hospitals whenever possible. In addition, the selected controls were children diagnosed with various diseases, in order to minimize the potential selection bias **(Pombo-de-Oliveira *et al*, 2006).** Additionally, controls were recruited among children of similar age as cases and presenting severe diseases aiming to restrain a possible recall bias. Another limitation is the difficulty of analyzing the frequency of use of each drug because such medicines are easily accessible in Brazil, they are cheap, not requiring a prescription, and improperly consumed.

On the other hand, this investigation has some strength. To our knowledge, this is so far the first investigation exploring the association between acetaminophen use during pregnancy and childhood leukemia in Latin-America population. Secondly, the

investigation focused on a specific age range (children 0-23 months), which has been scarcely addressed according to leukemia risk factors. Standardized procedures to obtain epidemiological data were employed, and participant enrollment was carried out following strict guidelines to minimize potential selection and recall biases.

In summary, this study presents suggestive evidence of a protective association between the use of maternal paracetamol use during pregnancy and ALL development in the offspring before the age of 1 year. Additionally, the study suggests a possible positive association between maternal use of dypirone during pregnancy and ALL risk offspring at the same age.

**APPENDIX**. List of pediatricians from *Brazilian Collaborative Study Group of Infant Acute Leukemia* who have contributed as co-authors to the study.

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## 6.3. Terceiro artigo da tese

# PREGNANCY, MATERNAL VITAMINS AND MINERAL SUPPLEMENTS INTAKE AND LEUKEMIA IN BRAZILIAN CHILDREN < 2 YEARS

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## Conflict of Financial Interests

The authors declare that there are not competing financial conflict of interests.

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**ABSTRACT**

**Background:** Some studies suggest that exposure to vitamin and mineral supplements can reduce the risk of developing childhood leukemia. However, results of previous epidemiological studies have been inconsistent.

**Methods**: A national hospital-based case-control study was carried out in Brazil in 1999-2007. Mothers of 252 cases and 423 controls 0-23 months residing in 13 states were interviewed. Information on maternal use of vitamins and minerals supplements 3 months before pregnancy, throughout pregnancy, and during breastfeeding was obtained. Unconditional logistic regression was used to estimate adjusted odds ratios (adj. ORs) for associations between maternal intake of these supplements and leukemia.

**Results:** Maternal vitamins and iron supplements consumption during pregnancy revealed decreased risk estimates of acute lymphocytic leukemia, ALL, respectively, adj.OR 0.33, 95% C.I. 0.12-0.92 and adj. OR 0.57, 95% C.I. 0.34-0.95. A possible protective effect following maternal either use of iron supplements or folic acid on ALL (OR 0.64, 95% C.I. 0.41-0.99) and acute myeloid leukemia, AML (OR 0.38, 95% C.I. 0.17-0.86) development were observed. Not statistically significant decreased risk estimates on the association between maternal use of vitamins and iron supplements and leukemia (cases with *MLL* gene rearrangements versus controls) in children < 1 yr were observed.

**Conclusion**: Vitamins and mineral supplements use during pregnancy may have a protective effect to leukemogenesis in the offspring < 2 yr.

**Keywords:** pregnancy, childhood leukemia; vitamins; iron, mineral supplements

## INTRODUCTION

Although rare, leukemia is the most diagnosed cancer in children under 15 years, representing about 30% of pediatric cases **(Linet, 1999).** Despite presenting debatable risk factors, it is believed that leukemia is associated with genetic alterations added to exposure to certain chemicals in the environment **(Greaves, 1999).** Some chemicals have a specific capacity to target the bone marrow via selective accumulation, may cause DNA damage and subsequently a leukemic genotype **(Wiemels, 2012).** Furthermore, one must consider that due to the short latency period of childhood leukemia, the mechanism of leukemogenesis may be due to sensitivity to the effects of potential damage to DNA during early pregnancy or in the first six months of the infant's life **(Greaves , 2003).**

Among the possible environmental risk factors associated to childhood leukemia, alcohol, tobacco, drugs, pesticides, organic solvents and ionizing radiation are included, the latter being the only acknowledged causal factor of leukemia, while the others are controversial **(Belson, 2007).** Among the possible protective factors for childhood leukemia, vegetables and fruits consumption and the use of mineral supplements and vitamins have been suggested **(Kwan *et al.,* 2009; Ross *et al.,* 2005).** A meta-analysis evaluated the use of multivitamins and the possible associations with different types of childhood cancers, observing a protective effect for leukemia (OR=0.61 95% C.I. 0.50-0.74), brain tumors (OR=0.73 95% C.I. 0.60-0.88) and neuroblastoma (OR=0.53 95% C.I. 0.42-0.68) **(Goh *et al.* 2007).**

The aim of this study was to evaluate the association between the use of vitamin and mineral supplements in the pre-conception, pregnancy and during lactation and the development of leukemia in the offspring < 2 years.

## METHODS

### Study population and design

This study is part of a multicentric investigation called "Multi-Institutional Study of Infant Leukemia: Contribution of Immunomolecular Markers in Distinguishing Different Etiopathogenic Factors", which focuses on the pathogenic mechanisms of early-age leukemia (EAL) in Brazil. The methodological characteristics of this multicentric study have been published elsewhere **(Mansur *et al*., 2009; Pombo-de-Oliveira *et al*., 2006).** Briefly, it consisted of a hospital-based case-control study, performed during 1999-2007, aiming to explore the different risk factors associated to EAL in Brazil. The study included participants recruited from institutions located in 13 states all over the country, excepting the North region. In particular, participants were recruited from hospitals in the cities of Belo Horizonte, Brasília, Campinas, Florianópolis, Goiânia, João Pessoa, Recife, Rio de Janeiro, Salvador, Santa Maria and São Paulo. Final study sample had a ratio of 2 controls per each EAL recruited case.

### Inclusion criteria

In the present study, the inclusion criteria were having been diagnosed of acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML) by morphological and immunophenotypical methods before the age of 2 years. Controls were selected among children of the same age range, hospitalized either in the same hospitals or in general hospitals located in the same municipality where the cases came from, and undergoing medical treatment for other non-malignant diseases **(Pombo-de-Oliveira *et al*., 2009).**

To avoid (or to minimize) selection bias, controls with diverse diagnosis were recruited. Hence, the probability of including patients with pathologies that have risk factors potentially associated with the exposure of interest was reduced **(Pombo-de-Oliveira *et al*., 2006).** Moreover, several life-threatening pathologies (infectious

diseases (29%); non-malignant hematological diseases (20%); respiratory diseases (10%); hemangioma (9%); diarrhea (9%); cardiovascular diseases (6%); others (17%)), were selected for the recruitment of controls aiming to also minimize recall bias.

**Exclusion criteria**

Cases and controls exclusion criteria were having adoptive parents, unknown or unavailable biological parents, myeloid dysplasia, and congenital syndromes. Patients diagnosed with malignant or benign tumors were also excluded from the control group, given the possible influence of environmental exposures in the etiology of any tumor. Additionally, cases presenting inconclusive diagnosis were also excluded.

**Data collection**

Data collection on environmental exposures potentially associated with leukemogenesis was performed through a specifically designed standardized questionnaire. Face to face interviews were carried out with mothers of cases and controls in order to obtain information about the socioeconomic background of the family, parents' occupational history, and lifestyle and health records of the family, including the use of drugs. Data regarding the use of vitamins and mineral supplements 3 months before pregnancy (pre-conception), during pregnancy and 3 months after childbirth (breastfeeding) were collected.

**Statistical analysis**

The statistical significance of the distribution of frequencies of the analyzed variables in cases and controls was determined using the chi-square test and Fisher's exact test (two-sided). Unconditional logistic regression was performed to ascertain the odds ratios (OR) for selected variables (birth weight, maternal education, maternal age,

contraceptive use, child skin color, and hormonal and pesticide exposure during pregnancy), and their respective 95% confidence intervals (C.I.), on the association between vitamins and mineral supplements use and EAL.

**Ethical Aspects**

This investigation was approved by the Ethics Research Committee of the Brazilian National Cancer Institute (CEP-INCA, 005/06) and the Ethical Committee of the National Public Health School, Oswaldo Cruz Foundation (CEP-ENSP, 126-638/2012). All case and control mothers participating in this study signed an informed consent form agreeing to participate.

**RESULTS**

This study was performed in a sample of 633 participants, including 227 cases (173 ALL and 54 AML) and 406 controls. The use of vitamins was reported by 40 (6.3%) mothers, 9 (22.5%) of which were EAL cases and 31 (77.5%) controls. Regarding mineral supplements, iron supplements use was reported by 182 (28.8%) mothers, 46 (25.3%) of which were EAL cases and 136 (74.7%). Folic acid use was reported by 23 (3.6 %) mothers, being 6 (26.1%) EAL cases and 17 (73.9%) controls. Socio-demographical data of participants revealed differences in the distribution of maternal age at birth, race, and education **(Table 1)**. In this sense, proportions of whites, older and more educated mothers among cases were significantly higher than in controls.

The results of this study presents a protective effect between the reported use of vitamins and development of ALL in children under the age of 2 years (OR=0.33, 95% C.I. 0.12-0.92), while the results for AML were not statistically significant (OR=0.62; 95% C.I. 0.17-2.23). Analyses stratified by gestational period despite presents a

protective effect in the three trimesters of pregnancy, were not statistically significant **(Table 2).**

We observed a protective effect for mothers who reported using iron supplements or folic acid and the development of ALL (OR=0.64, 95% C.I. 0.41-0.99) and AML (OR=0.38, 95% C.I. 0.17-0.86). Were also observed risk estimates with protective effect for ALL in the first trimester of pregnancy (OR=0.55, 95% C.I. 0.34-0.89) and for AML in the second (OR=0.34, 95% C.I. 0.14-0.85) and third trimester of pregnancy (OR=0.40, 95% C.I. 0.16-0.99) **(Table 3).**

After stratification by use of mineral supplements (iron supplement and folic acid), risk estimates were observed with a protective effect only for mothers who consumed exclusively iron supplementation in the first trimester (OR=0.57, 95% C.I. 0.34-0.95) and the development of ALL **(Table 4).** There were no statistically significant risk estimates at any gestational period for mothers who reported exclusive use of folic acid and even for mothers who consumed concomitantly iron supplements and folic acid **(Table 4).**

**Table 1**: Socio-demographic characteristics, leukemia cases and controls, children < 2 yr, Brazil, 1999-2007.

| | Cases | Controles | |
|---|---|---|---|
| | **n (%)** | **n (%)** | **p- value** |
| **Gender** | | | |
| Male | 122 (53.7) | 220 (54.2) | |
| Female | 105 (46.3) | 186 (45.8) | 0.94 |
| **Skin color** | | | |
| White | 121 (53.3) | 154 (37.9) | |
| No White | 106 (46.7) | 252 (62.1) | 0.01 |
| **Maternal age at birth (yr.)** | | | |
| <19 | 12 (5.3) | 72 (17.7) | |
| 19-24 | 78 (34.4) | 158 (38.9) | |
| 25-30 | 66 (29.1) | 100 (24.6) | |
| 31-36 | 52 (22.9) | 54 (13.3) | |
| >36 | 19 (8.4) | 22 (5.4) | 0.001 |
| **Maternal schooling (years)** | | | |
| <9 | 122 (53.7) | 279 (68.7) | |
| 9 or more | 105 (46.3) | 127 (31.3) | 0.001 |
| **Geographic origin** | | | |
| Southeast | 131 (57.7) | 223 (54.9) | |
| Northeast | 51 (22.5) | 99 (24.4) | |
| South | 28 (12.3) | 53 (13.1) | |
| Middle-West | 17 (7.5) | 31 (7.6) | 0.92 |
| **Total** | **227 (100.0)** | **406 (100.0)** | |

**Table 2:** Maternal exposure to vitamins during pregnancy, leukemia cases controls, children < 2 yr., Brazil, 1999-2007.

| Vitamins | Controls N (%) | ALL N (%) | ALL Crude OR (95% CI) | ALL Adj. OR* (95% CI) | AML N (%) | AML Crude OR (95% CI) | AML Adj. OR * (95% CI) |
|---|---|---|---|---|---|---|---|
| **Reported** | | | | | | | |
| No | 375 (92.4) | 168 (97.1) | 1.00 | 1.00 | 50 (92.6) | 1.00 | 1.00 |
| Yes | 31 (7.6) | 5 (2.9) | **0.36 (0.14-0.94)** | **0.33 (0.12-0.92)** | 4 (7.4) | 0.97 (0.33-2.86) | 0.62 (0.17-2.23) |
| **1st Trimester** | | | | | | | |
| No | 385 (94.8) | 169 (97.7) | 1.00 | 1.00 | 51 (94.4) | 1.00 | 1.00 |
| Yes | 21 (5.2) | 4 (2.3) | 0.43 (0.15-1.28) | 0.40 (0.12-1.29) | 3 (5.6) | 1.08 (0.31-3.74) | 0.71 (0.16-3.18) |
| **2nd Trimester** | | | | | | | |
| No | 385 (94.8) | 170 (98.3) | 1.00 | 1.00 | 54 (100.0) | - | - |
| Yes | 21 (5.2) | 3 (1.7) | 0.32 (0.10-1.10) | 0.30 (0.08-1.12) | 0 (0.0) | | |
| **3rd Trimester** | | | | | | | |
| No | 390 (96.1) | 170 (98.3) | 1.00 | 1.00 | 53 (98.1) | 1.00 | 1.00 |
| Yes | 16 (3.9) | 3 (1.7) | 0.43 (0.12-1.50) | 0.41 (0.11-1.62) | 1 (1.9) | 0.46 (0.06-3.54) | 0.31 (0.03-3.37) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

**Table 3:** Maternal exposure to iron supplements or folic acid during pregnancy, leukemia cases controls, children < 2 yr., Brazil, 1999-2007.

| Iron supplements or Folic acid | Controls N (%) | ALL N (%) | ALL Crude OR (95% CI) | ALL Adj. OR* (95% CI) | AML N (%) | AML Crude OR (95% CI) | AML Adj. OR * (95% CI) |
|---|---|---|---|---|---|---|---|
| **Reported** | | | | | | | |
| No | 259 (63.8) | 132 (76.3) | 1.00 | 1.00 | 45 (83.3) | 1.00 | 1.00 |
| Yes | 147 (36.2) | 41 (23.7) | **0.55 (0.36-0.82)** | **0.64 (0.41-0.99)** | 9 (16.7) | **0.35 (0.17-0.74)** | **0.38 (0.17-0.86)** |
| **1st Trimester** | | | | | | | |
| No | 287 (70.7) | 144 (83.2) | 1.00 | 1.00 | 45 (83.3) | 1.00 | 1.00 |
| Yes | 119 (29.3) | 29 (16.8) | **0.49 (0.31-0.76)** | **0.55 (0.34-0.89)** | 9 (16.7) | **0.48 (0.23-1.01)** | 0.52 (0.23-1.19) |
| **2nd Trimester** | | | | | | | |
| No | 289 (71.2) | 140 (80.9) | 1.00 | 1.00 | 47 (87.0) | 1.00 | 1.00 |
| Yes | 117 (28.8) | 33 (19.1) | **0.58 (0.38-0.90)** | 0.64 (0.40-1.02) | 7 (13.0) | **0.37 (0.16-0.84)** | **0.34 (0.14-0.85)** |
| **3rd Trimester** | | | | | | | |
| No | 300 (73.9) | 142 (82.1) | 1.00 | 1.00 | 47 (87.0) | 1.00 | 1.00 |
| Yes | 106 (26.1) | 31 (17.9) | **0.62 (0.39-0.96)** | 0.67 (0.41-1.08) | 7 (13.0) | **0.42 (0.18-0.96)** | **0.40 (0.16-0.99)** |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

**Table 4:** Maternal exposure to iron supplements or folic acid during pregnancy, leukemia cases controls, children < 2 yr., Brazil, 1999-2007.

| | Controls N (%) | N (%) | ALL Crude OR (95% CI) | Adj. OR* (95% CI) |
|---|---|---|---|---|
| **Reported** | | | | |
| No | 259 (63.8) | 132 (76.3) | 1.00 | 1.00 |
| Folic acid only | 11 (2.7) | 4 (2.3) | 0.71 (0.22-2.28) | 0.52 (0.15-1.79) |
| Iron supplements only | 130 (32.0) | 35 (20.2) | **0.53 (0.34-0.81)** | 0.65 (0.41-1.03) |
| Both | 6 (1.5) | 2 (1.2) | 0.65 (0.13-3.28) | 0.70 (0.12-4.12) |
| **1st Trimester** | | | | |
| No | 287 (70.7) | 144 (83.2) | 1.00 | 1.00 |
| Folic acid only | 10 (2.5) | 2 (1.2) | 0.40 (0.09-1.84) | 0.28 (0.06-1.40) |
| Iron supplements only | 105 (25.9) | 25 (14.5) | **0.47 (0.29-0.77)** | **0.57 (0.34-0.95)** |
| Both | 4 (1.0) | 2 (1.2) | 0.99 (0.18-5.50) | 0.93 (0.14-6.21) |
| **2nd Trimester** | | | | |
| No | 289 (71.2) | 140 (80.9) | 1.00 | 1.00 |
| Folic acid only | 10 (2.5) | 4 (2.3) | 0.83 (0.25-2.68) | 0.56 (0.16-1.95) |
| Iron supplements only | 102 (25.1) | 27 (15.6) | **0.55 (0.34-0.87)** | 0.64 (0.39-1.05) |
| Both | 5 (1.2) | 2 (1.2) | 0.83 (0.16-4.31) | 0.79 (0.13-4.87) |
| **3rd Trimester** | | | | |
| No | 300 (73.9) | 142 (82.1) | 1.00 | 1.00 |
| Folic acid only | 9 (2.2) | 3 (1.7) | 0.70 (0.19-2.64) | 0.45 (0.11-1.82) |
| Iron supplements only | 92 (22.7) | 26 (15.0) | **0.60 (0.37-0.96)** | 0.68 (0.41-1.15) |
| Both | 5 (1.2) | 2 (1.2) | 0.84 (0.16-4.41) | 0.87 (0.14-5.44) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide exposure and hormonal intake during pregnancy

**Table 5:** Maternal exposure to vitamins and iron supplements during pregnancy, *MLLr* cases and controls, children < 1 yr., Brazil, 1999-2007.

| | Controls n (%) | MLLr n (%) | Crude OR (95% CI) | Adj. OR (95%) |
|---|---|---|---|---|
| **Iron supplements** | | | | |
| No | 270 (66.5) | 44 (72.1) | 1.00 | 1.00 |
| Yes | 136 (33.5) | 17 (27.9) | 0.77 (0.42-1.39) | 0.87 (0.46-1.65) |
| **1st Trimester** | | | | |
| No | 297 (73.2) | 47 (77.0) | 1.00 | 1.00 |
| Yes | 109 (26.8) | 14 (23.0) | 0.81 (0.43-1.53) | 0.88 (0.44-1.74) |
| **2nd Trimester** | | | | |
| No | 299 (73.6) | 49 (80.3) | 1.00 | 1.00 |
| Yes | 107 (26.4) | 12 (19.7) | 0.68 (0.35-1.34) | 0.70 (0.34-1.43) |
| **3rd Trimester** | | | | |
| No | 309 (76.1) | 49 (80.3) | 1.00 | 1.00 |
| Yes | 97 (23.9) | 12 (19.7) | 0.78 (0.40-1.53) | 0.85 (0.42-1.74) |
| **Vitamins** | | | | |
| No | 375 (92.4) | 59 (96.7) | 1.00 | 1.00 |
| Yes | 31 (7.6) | 2 (3.3) | 0.41 (0.10-1.76) | 0.42 (0.10-1.90) |

*Adjusted for child skin color, maternal age, maternal education, birth weight, pesticide

## DISCUSSION

Some studies have reported the effects of the use of vitamin and mineral supplements in the development of childhood leukemia **(Ross *et al*., 2005, Kwan *et al.,* 2007; Goh *et al.* 2007).** Although the results of these studies are inconclusive, a biological plausibility supporting the hypothesis of a protective effect preventing carcinogenesis as a result of vitamins and mineral supplementation exists. Ames (2001) suggested that an inadequate intake of important micronutrients can contribute to DNA molecule damage, which may lead to oxidative stress, thus contributing to cancer development. These protective effects subsequently to vitamins and mineral supplementation could be explained by changes in DNA synthesis, repair and

methylation **(Kim, 1999).** Furthermore, it is noteworthy that the current hypothesis on leukemia etiology is based on the genetic and environmental factors interaction, being individual susceptibility partially conditioned by genetic polymorphisms **(Krajinovic *et al,* 2004).**

Methylenetetrahydrofolate reductase (MTHFR) enzyme plays an important role in folate metabolism, and either the differential activity from its variants (polymorphism) or changes in folate supplementation are differential its changes in activity (resulting from polymorphisms) can influence DNA synthesis, repair and methylation, and consequently, leukemogenesis susceptibility **(Krajinovic *et al,* 2004; Zanrosso *et al*, 2006).**

In this investigation, no association between exclusive use of folic acid and leukemia in children < 2 years was verified. Some authors commented that the analysis of the exclusive use of folate is unfeasible to be performed taking into account that its consumption is often associated with iron supplement intake **(Dockerty *et al.*, 2007).** Others have reported the difficulties of analyzing mineral compounds consumption separately due to the low prevalence of an exclusive use of each supplement **(Thompson *et al.,* 2001).**

Our results suggest a protective effect for the exclusive use of ferrous sulfate, being relevant to consider the low schooling level of participants in our study sample (Table 1). In Brazil, the distribution of ferrous sulphate by physicians to pregnant women, mainly those from low socioeconomical strata, is frequent. Hence, it can be hypothesized that participating mothers in this investigation probably had some difficulty to differentiate the use of ferrous sulfate and folic acid pills during pregnancy.

A possible protective effect among mothers reporting iron supplements or folic acid use according to ALL (OR=0.64, 95% C.I. 0.41-0.99) and AML (OR=0.38, 95% C.I. 0.17-0.86) in the offspring was observed. Lower ALL risk estimates were also

observed in the first trimester of pregnancy (OR=0.55, 95% C.I. 0.34-0.89) and for AML in the second and third trimesters, respectively, OR=0.34, 95% C.I. 0.14-0.85 and OR=0.40, 95% C.I. 0.16-0.99. Corroborating our results, other authors reported a protective effect to ALL in children < 15 yr. relatively to the use of iron supplements or folic acid, OR=0.37 95% C.I. 0.21-0.65 **(Thompson *et al.,* 2001).** Moreover, a study conducted in New Zealand where the prevalence of folate intake during pregnancy is low, no association between folate intake during pregnancy and ALL development was observed, OR=1.1, 95% C.I. 0.5-2.7 **(Dockerty *et al.,* 2007).**

After stratification by type of mineral supplements (iron supplement and folic acid) use, lower ALL risk estimates were only observed for mothers reporting exclusive iron supplementation use in the first trimester (OR=0.57, 95% C.I. 0.34-0.95).

An Australian study evaluating the exclusive consumption of iron reported a not statistically significant lower risk estimate, OR=0.75 95% C.I. 0.37-1.51 **(Thompson *et al.* 2001).** These authors also suggested that the incidence of childhood ALL decreased after the campaign to promote folate supplementation during pregnancy.

In our study, we observed a protective effect for ALL in the offspring of mothers who reported consuming vitamins, OR=0.33 95% C.I. 0.12-0.92. However, two studies did not find statistically significant lower risk estimates subsequently to the use of vitamins during pregnancy **(Shaw *et al,* 2004; Kwan *et al.* 2007).** A meta-analysis study conducted in Australia including 5 investigations reported a protective summary measure according to ALL in < 15 yr. subsequently to maternal vitamins intake before and during pregnancy, OR=0.83 95% C.I. 0.73-0.94 **(Milne et al., 2010).**

This study presents some limitations. As a hospital-based case-control study, selection and information biases could have occurred, and to prevent them, some procedures were adopted during research implementation. Hence, all participants were enrolled in the same cities, and in the same hospitals whenever feasible. Moreover,

controls were recruited among patients with different relatively risky conditions aiming to minimize selection and recall bias **(Pombo-de-Oliveira *et al*, 2006).** Additionally, it is important to consider that the use of vitamin and mineral supplements has a high prevalence during pregnancy in Brazil, which may minimize the misreporting use of such medication among participating mothers in our investigation.

Other limitation of this research is the lack of information on the dosage of vitamins or mineral supplements consumed during pregnancy. Information concerning the duration and frequency of these supplements use was unfeasible to be collected, thus restraining development of more detailed analyzes. Moreover, it was also unfeasible to obtain adjusted risk estimates on the use of vitamins according to relevant confounders such as maternal BMI or diet during pregnancy. Some authors have indeed previously suggested that proper analysis on the association between nutritional status and childhood leukemia should include a complete evaluation of maternal and infant diet, including vitamins supplementation **(Blair *et al.*, 2008).**

On the other hand, this investigation has some strength. So far, to our knowledge, this is the first investigation exploring the association between use of vitamin and mineral supplements in pregnancy and early age leukemia in Latin-America populations. Secondly, participants' enrollment was performed according to standardized criteria, as well as the adopted procedures to obtain epidemiological data in the interviews. Thirdly, the investigation analyzed such association focusing in a specific age range (children 0-23 months), generally poorly addressed elsewhere according to leukemia risk factors.

To conclude, the obtained results in this study aggregate additional evidence supporting the possible role of vitamins and mineral supplements consumption during pregnancy as a protective practice relatively to the development of childhood leukemia in the offspring.

**APPENDIX**. List of pediatricians from *Brazilian Collaborative Study Group of Infant Acute Leukemia* who have contributed as co-authors to the study.

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## 7. Considerações finais

Diante da hipótese de que a etiologia da leucemia seja decorrente da interação de fatores ambientais e/ou genéticos, esta tese de doutorado buscou explorar as informações epidemiológicas em um banco de dados referentes a estudo caso-controle nacional de base hospitalar. Esta investigação realizada em parceria com o INCa teve como objetivo avaliar a contribuição dos fatores hereditários e da exposição a medicamentos selecionados com o desenvolvimento de leucemia em menores de 2 anos.

No primeiro estudo foi avaliada a associação da história familiar de câncer (HFC) com a leucemia, observando resultados concordantes com a literatura internacional. Estes achados sustentam a hipótese de que a presença de antecedentes com HFC esteja associada ao processo de leucemogênese em crianças menores de 2 anos. Sendo assim, considerando a temática saúde pública, é importante que se proponha um acompanhamento diferenciado de tais grupos de alto risco, incluindo o rastreamento mais frequente e a avaliação clínica periódica destes indivíduos, possibilitando melhoria na organização das políticas de controle do câncer, incluindo aqueles para leucemia infantil.

Para o segundo e terceiro estudo, foi proposto avaliar a magnitude de associação entre o consumo de medicamentos no período gestacional, baseado na hipótese de que a exposição a substâncias químicas influenciam na suscetibilidade no desenvolvimento de leucemia na infância.

Para a realização do segundo estudo, foi avaliado os efeitos do consumo de analgésicos no período peri-gestacional e o desenvolvimento de leucemia em menores de 2 anos. Os resultados apresentaram evidências sugestivas de efeito protetor entre o uso de paracetamol durante a gestação e o desenvolvimento de leucemia em lactentes, enquanto os resultados obtidos em relação ao uso materno de dipirona apresentaram

associações positivas. Diante destes resultados, é importante ressaltar a necessidade de discussão da política de consumo de medicamentos no Brasil baseada na realidade das gestantes. Este fato decorre dos resultados de alguns estudos sugerindo que os mecanismos que levam ao desenvolvimento de patologias congênitas podem estar diretamente associados à toxicidade de substâncias químicas decorrente da exposição durante o período gestacional. Sendo assim, é regra geral que qualquer medicamento consumido no período gestacional deva ser ingerido em baixas doses terapêuticas, reduzindo o potencial risco de alterações adversas no desenvolvimento do concepto.

O terceiro estudo proposto na tese foi o de avaliar a magnitude de associação entre o uso de vitaminas e suplementos minerais no período gestacional e o desenvolvimento de leucemia em menores de 2 anos. Apesar de resultados divergentes encontrados na literatura internacional em relação a esta exposição, alguns estudos têm sugerido efeito protetor entre o uso de vitaminas e suplementos minerais e a leucemogênese infantil. O presente estudo também observou associações com efeito protetor entre o uso de vitaminas e suplemento de ferro durante a gestação e o desenvolvimento de leucemia em menores de 2 anos. É importante considerar, que o uso de vitaminas e suplementos minerais durante a gestação é uma intervenção de rotina no Brasil, porém, os órgãos de saúde deveriam estabelecer doses preconizadas de acordo com a necessidade de cada gestante.

Por fim, é importante ressaltar que na literatura, esta parece ser a primeira investigação realizada com crianças na América Latina explorando a hipótese de que a exposição a medicamentos durante a gestação, somada à contribuição de antecedentes familiares de câncer estejam associadas ao desenvolvimento de leucemia na infância. Adicionalmente, é preciso considerar que as leucemias pertencem a um grupo de patologias raras na infância, principalmente em menores de 2 anos, indicando assim a

necessidade de serem estudadas as interações genético-ambientais com intuito de ampliar o conhecimento acerca da etiologia das leucemias na infância.

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