UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

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O IMPACTO DA ANEMIA E DOENÇA FALCIFORME DURANTE A GESTAÇÃO

THE IMPACT OF ANEMIA AND SICKLE CELL DISEASE DURING PREGNANCY

CAMPINAS

2021

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Tese apresentada à Faculdade de Ciências Médicas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências da Saúde, área de concentração Saúde Materna e Perinatal

Doctoral Thesis presented by the Graduate Studies, Faculty of Medical Sciences, State University of Campinas – UNICAMP to obtain the title of Doctor of Health Sciences, concentration area Maternal and Perinatal Health

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ESTE TRABALHO CORRESPONDE À VERSÃO
FINAL DA DISSERTAÇÃO/TESE DEFENDIDA PELA
ALUNA CAMILLA OLIVARES FIGUEIRA, E ORIENTADA PELA
PROFA. DRA. MARIA LAURA COSTA DO NASCIMENTO

CAMPINAS

2021

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

F469i

Figueira, Camilla Olivares, 1982-
O impacto da anemia e doença falciforme durante a gestação / Camilla Olivares Figueira. – Campinas, SP : [s.n.], 2021.

Orientador: Maria Laura Costa do Nascimento.
Coorientador: Fernanda Garanhani de Castro Surita.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Doença falciforme. 2. Gestação. 3. Anemia. 4. Morbidade materna. 5. Morbimortalidade neonatal. I. Nascimento, Maria Laura Costa do, 1979-. II. Surita, Fernanda Garanhani de Castro, 1964-. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: The impact of anemia and sickle cell disease during pregnancy
Palavras-chave em inglês:
Sickle cell disease
Pregnancy
Anemia
Maternal morbidity
Neonatal morbidity and mortality
Área de concentração: Saúde Materna e Perinatal
Titulação: Doutora em Ciências da Saúde
Banca examinadora:
Maria Laura Costa do Nascimento [Orientador]
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Sérgio Hofmeister de Almeida Martins Costa
Evelyn Traina
Data de defesa: 28-09-2021
Programa de Pós-Graduação: Tocoginecologia

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-1412-8094
- Currículo Lattes do autor: http://lattes.cnpq.br/8449012880177834

COMISSÃO EXAMINADORA DA DEFESA DE DOUTORADO

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Data de Defesa: 28/09/2021

Agradecimentos

Á minha mãe e irmã pelo amor incondicional.

Ao Renato, meu marido por entender e apoiar minhas ausências e aos meus filhos Marcella e Felipe, por quem sempre tentarei ser melhor a cada dia.

À Profa. Dra. Maria Laura Costa do Nascimento, minha orientadora, pelo incentivo, disponibilidade e por confiar sempre na conclusão desse trabalho.

À Profa. Dra. Fernanda Garanhani de Castro Surita, minha coorientadora, pela ajuda durante todo o projeto.

Aos amigos Anderson, Tábata, José Paulo e Kleber que foram de ajuda inestimável em momentos importantes na confecção da tese.

Aos pacientes que se disponibilizam a participar de estudos científicos e contribuem anonimamente para a melhora na qualidade da assistência e conhecimento.

RESUMO

Introdução: Anemia (definida como Hb<11 g/dL) é condição muito frequente durante a gestação, associada a piores desfechos gestacionais e maior risco de prematuridade. Dentre as anemias de causa congênita, a doença falciforme é a mais comum, cursando com anemia hemolítica, elevada morbidade e mortalidade materna e perinatal. **Objetivos:** Avaliar prevalência de anemia na gestação a termo e pré-termo, resultados maternos e perinatais nesses subgrupos e nas portadoras de doença falciforme, comparando esses resultados nos diferentes genótipos da doença e achados anatomopatológicos placentários. **Métodos:** Foram realizados 3 estudos: 1) análise secundária do banco de dados do Estudo Multicêntrico de Investigação de Prematuridade (EMIP), visando esclarecer os desfechos maternos e perinatais de gestantes com anemia e comparando esses resultados no parto a termo e pré-termo. Para comparação das variáveis categóricas foi usado o teste Qui quadrado ou exato de Fisher. Para as variáveis numéricas, utilizado Mann Whitney e para avaliação de associação independente das variáveis com anemia no termo e pré-termo foi realizada análise multivariada; 2) revisão narrativa de literatura acerca da doença falciforme. Realizada tabela descritiva com os achados do estudo; 3) estudo de coorte retrospectivo, que incluiu 62 mulheres com doença falciforme acompanhadas no pré-natal especializado, na Universidade Estadual de Campinas, entre 2011 e 2017, com avaliação de complicações maternas e perinatais e descrição dos achados anatomopatológicos placentários. Foi realizada uma análise descritiva, com prevalência dos três genótipos mais frequentes de doença falciforme, resultados maternos, perinatais e descrição da morfologia placentária quando disponível. Para as variáveis numéricas foram utilizados os testes Mann Whitney ou Kruskal-Wallis dependendo se a comparação foi entre duas ou três variáveis respectivamente. As variáveis categóricas foram divididas em grupos e apresentadas em percentual (%) de frequência. **Resultados:** 1) prevalência geral de anemia foi de 31.7%, sendo 27.74% no termo e 33.23% no grupo pré-termo (p<0.01). Os fatores associados com parto pré-termo e anemia foram: idade materna abaixo de 19 anos (p= 0.047), cor da pele não branca (p= 0.001), escolaridade abaixo de 8 anos (p= 0.003), filho abaixo de 5 anos (p= 0.001), início tardio do pré-natal (p= 0.001), menos de 6 consultas de pré-natal (p= 0.007), morbidade neonatal (p= 0.001) e suporte ventilatório neonatal (p= 0.003). 2) revisão da literatura mostrou maior risco de piora da anemia, infecções,

complicações pulmonares, pré-eclâmpsia, morte materna e neonatal, baixo peso ao nascer, restrição de crescimento fetal nas gestantes com doença falciforme. Infecção urinária, cor da pele não branca, menos de 6 consultas de pré-natal se associaram de forma independente a anemia nos partos pré-termo. 3) Foram acompanhadas 62 gestantes com doença falciforme no período de 2011 a 2017, sendo 25 com genótipo HbSS, 29 com genótipo HbSC e 8 HbSβ. No geral aumentaram as taxas de parto prematuro (32%), parto cesariana (79%), near miss (22%) e complicações da doença. As portadoras da doença homozigótica (HbSS) apresentaram maior índice e gravidade de complicações quando comparado às formas heterozigóticas. **Conclusão:** Anemia em gestantes no Brasil é prevalente, sendo fator de risco para prematuridade. A doença falciforme é uma condição com alto risco de complicações maternas e perinatais e necessita de seguimento multidisciplinar adequado.

Palavras chave: doença falciforme, gestação, anemia, morbidade materna, morbidade neonatal.

ABSTRACT

Background: Anemia (defined as Hb level <11g/dl) is a very common condition at pregnancy and is associated to worse pregnancy outcomes e higher prematurity rates. Among genetic causes of anemia, sickle cell disease (SCD) is the most common, presenting with hemolytic anemia and enhancing maternal and perinatal morbidity and mortality. **Objective**: To evaluate the prevalence of anemia in term and preterm pregnancies; maternal and perinatal outcomes in women with SCD, comparing the different genotypes and to describe morphological placental findings. **Methods:** Three studies were performed: 1) Secondary analysis of the Brazilian multicenter Study on Preterm Birth to describe maternal and perinatal outcomes in term and preterm birth of pregnant women with anemia. For comparison of categorical variables, Chi square or Fisher's exact test was used if necessary. For numerical variables, Mann Whitney was used, and for evaluation of independent association of the variables with anemia in term and preterm pregnancies, a multivariate analysis was performed; 2) Review of literature on SCD with a descriptive table of findings. 3) Retrospective cross-sectional study with 62 women with SCD followed at the specialized prenatal care of the University of Campinas between 2011 and 2017, evaluating maternal and perinatal outcomes and describing abnormal placental findings. A descriptive analysis was carried out, with prevalence of the different sickle cell genotypes, maternal, perinatal results and description of the placental morphology when available. Mann Whitney or Kruskal-Wallis was performed for statistical analysis of Numerical variables if the comparison was between two or three groups respectively. Categorical variables were divided into groups and presented as a percentage (%) of frequency. **Results:** 1) Overall anemia prevalence was 31.7%. At term it was 37.74% and 33.23% among the preterm group ($p<0.01$). Factors associated with preterm birth and anemia were: maternal age ≤19 years ($p=0.047$), non-white skin color ($p=0.001$), ≤ 8 schooling years ($p=0.003$), the presence of previous children under 5 years old ($p=0.001$), late onset of prenatal care ($p=0.001$), <6 prenatal care visits ($p=0.007$), neonatal morbidity ($p= 0.001$) and ventilator support ($p= 0.003$). 2) Review of literature showed higher risk of worsening anemia, infections, pulmonary complications, preeclampsia, maternal and neonatal death, low birth weight, fetal growth restriction in women with SCD. Urinary tract infection, nonwhite skin color, less than six antenatal visits were independently associated to anemia in preterm births. 3) Sixty-two pregnant women with SCD were

followed from 2011 to 2017, 25 HbSS genotype, 29 HbSC genotype and 8 HbSβ. Overall there was increased rates of preterm birth (32%), cesarean section (79%), maternal nearmiss (22%) and complications of the SCD. The homozygous form of the disease (HbSS) had more frequent and severe complications when compared to heterozygous forms of the disease. **Conclusions:** anemia in pregnant women is a significant risk factor for prematurity, and sickle cell disease is a condition with high risk of maternal and perinatal adverse outcomes and therefore, should trigger multidisciplinary follow up.

Keywords: sickle cell disease, anemia, pregnancy, maternal morbidity, neonatal morbidity.

TABELAS E FIGURAS

Artigo 1

Artigo 2

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LISTA DE ABREVIATURAS E SIGLAS

ACE: angiotensin-converting enzyme

ACS: acute chest syndrome

AVC: acidente vascular cerebral

BMI: body mass index

CAEE: Certificado de Apresentação de Apreciação Ética

CAISM: Centro de Atenção Integral a Saúde da Mulher

CEP: Comitê de Ética em Pesquisa

CONEP: Comitê Nacional de Ética em Pesquisa

CS: cesarean section

DEXA: dual-energy X-ray absorptiometry

dL: decilitro

DP: desvio padrão

EMIP: Estudo Multicêntrico de Investigação de Prematuridade

EUA: Estados Unidos da América

FGR: Fetal Growth Restriction

g: grama

GA: gestational age

HELLP: hemolysis, elevated liver enzymes, and low platelets

Hb: hemoglobina

HbA: hemoglobina A

HbS: hemoglobina S

HbSβ: heterozigose hemoglobina S com cadeia β hemoglobina

HbSC: heterozigose hemoglobina S com hemoglobina C

HbSS: hemoglobina S em homozigose (anemia falciforme)

IG: idade gestacional

Kg: quilogramas

LBW: low birth weight

LDH: lactate dehydrogenase

M: média

m^2: metro quadrado

mg: miligramas

mm: milímetros

MM: maternal mortality

N: número

ND: neonatal death

O2: oxigênio

OMS: Organização Mundial de Saúde

OR: odds ratio

p: probabilidade de significância

PE: preeclampsia

pPROM: preterm premature rupture of membranes

PTB: preterm birth

RBC: red blood cell

RCF: restrição de crescimento fetal

SAS: Statistical Analisys System

SCD: sickle cell disease

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

TCLE: Termo de Consentimento Livre e Esclarecido

US: ultrassonografia

UTI: unidade de terapia intensiva

UTI: urinary tract infection

VOC: vasooclusive crisis

WA: worsening anemia

WHO: World Health Organization

$X^{2:}$ qui quadrado

%: percentual

Sumário

1.INTRODUÇÃO

1.1 Anemia e Gestação

Anemia é definida pela Organização Mundial de Saúde (OMS) como a condição na qual o nível de hemoglobina está abaixo de um valor considerado normal, podendo ser devido à carência de um ou mais nutrientes essenciais. É um importante problema de saúde pública, pois afeta mais de 2 bilhões de pessoas em todo mundo (1) e acomete pessoas de todas as classes sociais tanto em países de alta como baixa renda, especialmente mulheres e crianças (2-4). Estima-se que a prevalência em gestantes seja em torno de 40% de acordo com a OMS (5). As gestantes são um grupo propenso a desenvolver anemia, pois além do aumento da demanda de nutrientes pelo feto e na amamentação, há ainda a hemodiluição e perda sanguínea esperada no parto. Na gestação, anemia é definida como níveis de hemoglobina abaixo de 11g/dL em qualquer fase da gestação (5, 6) e estudos sugerem que a anemia é fator de risco para prematuridade, ruptura prematura de membranas, baixo peso ao nascer, aumento de morbidade materna e neonatal, necessidade de transfusão sanguínea, infecções, hipertensão gestacional e pré-eclâmpsia (6, 7).

No Brasil, estudos estimam que a prevalência de anemia chegue a 32 (8) a 35% (9), porém varia de acordo com a região e é diretamente dependente da subnotificação de casos. Em adolescentes a prevalência estimada é maior e chega a de 41%, provavelmente por maior déficit nutricional (10). Como medida preventiva, a OMS recomenda a suplementação de 30 a 60mg de ferro elementar desde o início da gestação (4).

Importante averiguar a causa da anemia durante a gestação, a fim de otimizar o tratamento. As causas mais comuns são: a anemia fisiológica que ocorre especialmente no 3º trimestre, deficiência de ferro, deficiência de vitamina B12 e folato e condições genéticas, incluindo a doença falciforme.

1.2 Doença falciforme

A doença falciforme é uma das alterações genéticas conhecidas mais comuns na população mundial e supõe-se que tenha surgido no continente africano há mais de um século e se espalhado pelo restante do mundo através do trabalho escravo (11). Anualmente, acomete mais de 300.000 nascidos vivos no mundo (12-14) e sua prevalência nos continentes reflete a prevalência do gene mutado circulante na população. Nas Américas a taxa de bebês nascidos com doença falciforme chega a 0.49 por 1000 nascidos, 0.07 por 1000 na Europa, 0.68 por 1000 no sul e sudeste Asiático e 10.68 por 1000 no continente Africano (15). Dados nacionais estimam cerca de 2 milhões de portadores do gene HbS e 25 a 50000 acometidos com a forma homozigótica e mais grave da doença (16).

A doença falciforme é uma desordem genética autossômica recessiva com mutação na cadeia S da hemoglobina e pode se apresentar na forma homozigótica, ou seja, com as duas cadeias S da hemoglobina mutadas (HbSS), condição conhecida como anemia falciforme. Ou então essa cadeia S mutada pode se combinar com diferentes variações da hemoglobina, ocasionando as formas heterozigóticas, incluindo: HbSC quando em associação com a hemoglobina C, HbSβ, quando em associação com alterações na cadeia beta – traço talassêmico (HbS/beta talassemia) (11, 12, 17). Esses genótipos heterozigóticos têm, em geral, uma evolução mais benigna (18, 19), porém ainda

são sujeitos a complicações ao longo da vida, especialmente no ciclo grávido-puerperal.

A alteração da hemoglobina na doença falciforme consiste na polimerização da deoxihemoglobina S que, quando submetida a condição de baixa tensão de O2, altera a membrana do glóbulo vermelho tornando-o menos flexível e mais aderente às células endoteliais e favorecendo o estado pró coagulante (20). Essas alterações podem ocorrer inclusive nos reticulócitos (21). Assim, o glóbulo vermelho além de perder sua função de transporte de oxigênio, causando hipóxia, é retirado da circulação tanto pelo sistema retículo endotelial como por hemólise intravascular (22). A liberação de hemoglobina livre na circulação e a maior aderência das células endoteliais leva a ativação endotelial, produção de citocinas e ativação da cascata de coagulação gerando uma resposta inflamatória crônica e sistêmica (23). Neutrófilos e plaquetas também são ativados, culminando em vaso-oclusão (21, 22) com lesão de isquemia/ reperfusão (20, 24). Assim, a doença em geral, se caracteriza por anemia hemolítica crônica e crises vaso oclusivas (17, 20) com lesão de órgãos por isquemia e inflamação (22).

Por se tratar de uma condição capaz de acometer múltiplos órgãos, está associada a alta morbidade (12, 18, 25-27) e mortalidade precoce (18, 26, 28). A análise dos dados de mortalidade na doença falciforme entre 1979 e 2005 realizado nos EUA encontrou que a expectativa de vida era, em média, 38 anos para homens e 42 anos para mulheres, sendo a gravidez o fator de maior impacto para a morte dessas mulheres (29). Porém observou-se que alguns pacientes com doença falciforme atingiam idades mais avançadas, e concluiu-

se que a doença afeta seus portadores com intensidades variadas, levando a discrepância nos dados acerca de expectativa de vida. Platt et al., encontrou em 1994 que a idade mediana de sobrevida dos pacientes com anemia falciforme era 42 anos para homens e 48 anos para mulheres (30). Apesar da discrepância dos dados, sabe-se que a expectativa de vida é reduzida em seus portadores.

Nos últimos 30 anos, os avanços nos estudos proporcionaram melhor conhecimento da doença, fisiopatologia e reconhecimento precoce de suas complicações. Intervenções precoces ainda na infância como o uso de penicilina, vacinação, rastreamento e prevenção de acidente vascular cerebral, melhoraram a qualidade de vida para os portadores da doença e aumentaram a sobrevida, contribuindo assim para que muitas mulheres atingissem a idade reprodutiva (31-34) e engravidassem. Entretanto, as gestações em portadoras de doenças crônicas tendem a ser de maior risco e necessitar de atenção pré-natal mais cuidadosa. Essa maior incidência de complicações maternas e fetais na doença falciforme (27, 35-39) suscita que o seguimento seja realizado por uma equipe multidisciplinar treinada, contudo, nas doenças falciformes, os diversos fenótipos e nuances na apresentação da doença limitam a previsibilidade da evolução das gestações (40).

As complicações da doença falciforme são inúmeras, entre as quais: anemia hemolítica, crises vaso oclusivas (a oclusão compromete o fluxo sanguíneo local, proporcionando maior estase do sangue e aumento da sua viscosidade, culminando em novas oclusões) (41), maior susceptibilidade a infecções, (particularmente infecção do trato urinário), eventos trombóticos (tromboembolismo venoso, acidente vascular cerebral), síndrome torácica aguda

(principal causa de mortalidade tanto na fase aguda como crônica por deterioração progressiva da função pulmonar), comprometimento renal crônico (11). Já as complicações fetais incluem restrição de crescimento fetal, baixo peso ao nascer, prematuridade, sofrimento fetal durante o trabalho de parto e aumento da mortalidade perinatal (18, 26, 27, 38, 42). E essas complicações podem ser extremamente graves se não identificadas e manejadas rapidamente, tornando imprescindível seu diagnóstico precoce a fim de reduzir a morbidade e mortalidade pela doença. Os dados sobre mortalidade materna são muito amplos na literatura, variando de 1% em um estudo retrospectivo realizado no Reino Unido (25) até 9,2% em um estudo Nigeriano (43). Essa discrepância pode ocorrer por prevalência variável da doença e consequente habilidade em lidar com suas complicações, bem como por subnotificação dos casos. Em 2015, uma metanálise com mais de 26 mil mulheres concluiu que as pacientes com doença falciforme têm o dobro de risco de desenvolverem pré-eclâmpsia ao longo da gestação e aquelas com genótipo HbSS apresentam quase cinco vezes mais risco de apresentarem eclâmpsia (18).

As mulheres que sobreviveram a eventos adversos graves no ciclo grávido puerperal passaram a ser ponto de grande interesse nos estudos, a fim de melhorar o conhecimento e identificação de complicações potencialmente graves e dos fatores que contribuem para mortalidade materna. Dessa forma, a Organização Mundial de Saúde nomeou o conceito de *near miss*. Esse conhecimento permite identificar os casos potencialmente fatais, intervir precocemente e, portanto, melhorar tanto a saúde materna como as sequelas que podem advir dessas complicações e reduzir a mortalidade (44).

A morbidade materna grave ou *near miss* é um problema de saúde pública na América Latina e é definida pela OMS como sendo um evento de quase morte de uma mulher por complicações durante a gestação, parto ou durante os 42 dias após o término da gestação. Os fatores que mais se relacionaram com morte materna foram: admissão em unidade de terapia intensiva (UTI), transfusão de sangue, histerectomia, ocorrência de eclâmpsia e complicações renais e/ou cardíacas. Ainda de acordo com Souza, JP et al., em 2010 na América Latina, ocorriam 34 casos de *near miss* para cada 1000 nascimentos (45) e estima-se que para caso de morte materna ocorram 15 casos de *near miss* (46, 47). Tornou-se um tema frequente nos estudos de pacientes com doenças graves e no caso das doenças falciformes até 1/3 das pacientes podem apresentar essa condição de *near miss*, sendo que os eventos mais frequentes são pneumonia/ complicações pulmonares e síndrome torácica aguda (26, 48). As doenças falciformes aumentam a morbidade do período gestacional e puerpério e com isso aumentam a chance de uma paciente apresentar um evento *near miss*, tornando a atenção pré e pós natal um período crítico na vida dessas pacientes. Além da gravidade do acometimento materno e ameaça à vida da paciente, observou-se que a ocorrência de um evento *near miss* também está associada a recém-nascido de baixo peso e muito baixo peso, admissão em UTI neonatal, óbito fetal, morte neonatal precoce e hospitalização materna prolongada (45, 49).

A gestação de mulheres com doença falciforme necessita de seguimento multidisciplinar frequente e adequado (27, 39, 50) incluindo consultas pré-natais, avaliação nutricional, suporte psicológico, avaliações por equipe de hematologia, exames laboratoriais pertinentes à patologia e acompanhamento fetal rigoroso,

pois além de apresentarem maior risco de complicações como pré-eclâmpsia, eclâmpsia e piora da anemia, o feto dessas mulheres também apresenta risco maior de evoluir com restrição de crescimento (RCF), baixo peso, óbito fetal (18, 26, 27, 51) e alterações na vitalidade que podem culminar na necessidade de antecipação do parto, aumentando a morbidade neonatal quando comparada com mulheres com perfil normal de hemoglobina. O comprometimento fetal pode ocorrer tanto pelos baixos níveis de hemoglobina causado pela própria anemia característica da doença, como também por alterações placentárias devido a episódios recorrentes de vaso-oclusão e alteração inflamatória crônica. O genótipo HbSS é o de maior risco para complicações maternas e fetais/ neonatais (18, 26, 51). A anemia materna é uma condição comumente encontrada em gestantes, especialmente no último trimestre em que a necessidade fetal de ferro aumenta, depletando os estoques maternos (52). Essa condição é mais prevalente em países em desenvolvimento devido a questões econômicas e nutricionais (53). Sabe-se que a anemia materna no 1º e 2º trimestre da gestação é um dos fatores responsáveis por recém-nascidos de baixo peso, prematuridade, complicações e morte neonatais (52, 54, 55).

Uma das características da doença falciforme é a presença de anemia crônica, normalmente presente já no início da gestação. Dependendo da gravidade da anemia nessa fase, pode comprometer negativamente o desenvolvimento fetal, levando a RCF (19, 52, 53, 56). Além da anemia, outras características e complicações da doença falciforme podem interferir no potencial de crescimento do feto como alterações placentárias que levam a hipóxia (20, 53, 57), pré-eclâmpsia, eclampsia e prematuridade (18, 58).

A ultrassonografia (US) é o exame capaz de suspeitar dessas alterações no desenvolvimento fetal e desde sua introdução na prática clínica em 1958 (59), houve melhora na detecção de complicações fetais, em especial das situações relacionadas com alterações hemodinâmicas e de seu bem estar. Com o surgimento do Doppler (que consiste na percepção da variação do som de um objeto em movimento e, portanto, possibilitou a avaliação das áreas em movimento na ultrassonografia, particularmente as hemácias nos vasos), o estudo hemodinâmico do feto melhorou sensivelmente. O termo *hemodinâmica* refere-se basicamente ao estudo de como o organismo se adapta para a adequada oxigenação dos tecidos (60) e sua introdução na prática ultrassonográfica data de 1977 com o estudo da Dopplervelocimetria das artérias umbilicais (61). Com o avanço nos estudos e na melhora técnica os aparelhos de US, outros vasos passaram a ser estudados e correlacionados com o bem estar fetal como a artéria cerebral média, istmo aórtico, ducto venoso e artérias uterinas.

No geral, as pacientes portadoras de doenças crônicas são as mais susceptíveis a apresentarem algum acometimento fetal e, portanto, as que devem manter seguimento mais frequente, incluindo as pacientes com doença falciforme. Com isso, a US é fundamental no acompanhamento do bem estar fetal dessas gestantes, permitindo tanto o diagnóstico de possíveis complicações fetais, como o seguimento das mesmas (53), visando detectar o momento oportuno de nascimento e minimizar as consequências advindas da prematuridade. Além da detecção de alterações fetais, a US pode identificar também as mulheres com maior risco de apresentarem complicações hipertensivas e prematuridade através de rastreamento em épocas específicas

da gestação. Assim, a US tornou-se uma ferramenta indispensável na rotina pré-natal dessas pacientes.

A maioria dos estudos envolvendo seguimento US de pacientes com doença falciforme prioriza a avaliação da estimativa de peso fetal e sua comparação com pacientes sem alterações de hemoglobina (62, 63). Observou-se que os fetos daquelas mães apresentavam menor peso fetal estimado no US gestacional que se correlacionou com maior frequência de neonatos com baixo peso ao nascimento (19, 26, 58). Porém essa percepção de menor peso estimado à US muitas vezes só é significativa e notada no terceiro trimestre da gestação (62), ficando a US com o papel de seguimento e monitoramento da condição fetal, já que nesses casos têm pouco efeito as ações preventivas. Assim, parâmetros Dopplervelocimétricos podem ser estudados mais detalhadamente nas pacientes com doença falciforme e que apresentam risco aumentado para RCF, a fim de suspeitar precocemente dessa condição e programar acompanhamento.

A RCF é a condição em que o feto não atinge seu potencial de crescimento e normalmente está relacionada com função placentária deficiente (64). Esses fetos apresentam maior risco de complicações ao longo da gestação, maior morbidade e mortalidade e prejuízo no desenvolvimento neurológico e cognitivo (65, 66), sendo, portanto, muito importante a detecção desses casos e seguimento apropriado.

É comumente definida como desvios de peso em relação a curvas de peso populacionais (geralmente abaixo do percentil 10) (67) construídas com base em população sem patologias conhecidas, porém essa definição ignora parâmetros

funcionais, subestimando os casos de RCF em que o peso fetal se encontra acima do percentil 10 e superestima casos em que apesar do peso abaixo desse marco não há alteração funcional (68). A anemia crônica é uma das características da doença falciforme e uma das possibilidades para um menor ganho de peso dos fetos. Como o nível de hemoglobina pode chegar a valores muito baixos, muitas pacientes necessitam de transfusões sanguíneas ao longo da gestação. Com isso, surgiram estudos avaliando o benefício de transfusões profiláticas, que consistem em transfusões sanguíneas programadas a partir de uma idade gestacional pré estabelecida (usualmente no 3º. trimestre, porém ainda sem consenso global do melhor momento para seu início) evitando que o nível de hemoglobina dessas pacientes alcançasse valores críticos. Em 2015 uma metanálise concluiu que as transfusões profiláticas reduziram a mortalidade materna, os episódios vaso oclusivos, as complicações pulmonares incluindo os casos de embolia, os episódios de pielonefrite, a mortalidade perinatal e neonatal e a taxa de parto prematuro. Porém não teve impacto na prevalência de pré-eclâmpsia e RCF, sugerindo que a etiologia mais provável dessa última seria insuficiência placentária. Essa metanálise avaliou os dados de 12 estudos e desses, dois realizaram transfusões no início até o meio da gestação e nove deles teriam grande risco de bias (69). Outros estudos corroboram a hipótese de que a realização de transfusões profiláticas em vez de realizar somente quando necessário por reduzir não só a mortalidade materna (70) como também os episódios vaso oclusivos (70, 71). Por outro lado, o ensaio clínico randomizado realizado por Koshy et al., não encontrou diferença na mortalidade perinatal, óbito fetal e neonatal, porém houve diferença significativa nos episódios vaso oclusivos (72, 73). Esse estudo, entretanto, avaliou apenas 36 casos e foi

realizado em 1988 e desde então houve muito avanço nos cuidados neonatais e procedimentos transfusionais, sendo difícil compará-lo a estudos mais recentes. Além disso, a época de início das transfusões profiláticas não é bem definida na literatura, sendo que a maioria se inicia no segundo trimestre da gestação (70) e alguns estudos realizaram a intervenção na primeira metade da gravidez (73, 74).

A doença falciforme normalmente requer transfusões de sangue ao longo da vida e na gravidez a tendência é de que a necessidade de transfusão aumente por piora da anemia e maior risco de complicações. Apesar dos procedimentos transfusionais serem bastante seguros atualmente, há o risco de aloimunização, que consiste no surgimento de anticorpos contra antígenos presentes nas hemácias doadas. No geral as taxas de aloimunização são próximas de 16-20% (39, 70). A presença desses anticorpos na circulação pode dificultar o encontro de sangue compatível para futuras transfusões, especialmente se a necessidade de transfusão não for esporádica, como ocorre nos casos de transfusão profilática das gestantes. Por outro lado, como o procedimento é programado haveria maior tempo para encontrar o sangue com a maior compatibilidade possível, sendo que nas transfusões realizadas com certa urgência, muitas vezes não há tempo hábil para encontrar o sangue ideal.

Outro fator que contribui para o déficit no ganho de peso desses fetos durante a gestação são as alterações placentárias das gestantes com doença falciforme. Apesar de ainda não ser totalmente conhecido o mecanismo pelo qual o desenvolvimento placentário dessas pacientes pode interferir negativamente no resultado perinatal e surgimento de complicações, um estudo realizado em

2016 por Melo e cols. mostrou que há expressão de vários genes pró-inflamatórios na placenta dessas pacientes, sugerindo que seja um ambiente exposto a alterações inflamatórias crônicas e hipóxia (20). Esse desequilíbrio com predomínio de substancias pró inflamatórias favoreceria a ocorrência de episódios vaso oclusivos e necrose (57, 75), reduzindo a porção efetivamente funcionante da placenta, podendo ser o responsável por complicações da doença como RCF, pré-eclâmpsia, e parto prematuro (20). Esse estudo encontrou redução significativa do peso fetal das pacientes com anemia falciforme (HbSS) e idade gestacional mais precoce de nascimento. Comparou ainda as complicações entre os genótipos HbSS e HbSC encontrando com maior frequência episódios de vaso oclusão, RCF, mortalidade perinatal e admissão em UTI nas pacientes HbSS (20). Entretanto, o estudo de Trampont et al., ao avaliar as placentas das pacientes com doença falciforme, não observou alterações sugestivas de processo inflamatório, mas encontrou alterações como nós sinciciais, depósito excessivo de fibrina, necrose de vilos e congestão (57). Ambos os estudos concluíram que as apesar das alterações no funcionamento placentário, não houve diferença nos pesos das placentas entre as pacientes com e sem doença falciforme. A avaliação histológica da placenta é passível de muitas alterações, dificultando a padronização dos achados nos estudos e também a comparação dos resultados encontrados em diferentes estudos. Em 2012, Turowski e cols estudaram 315 placentas advindas de óbitos fetais e patologistas e obstetras propuseram uma classificação unificada das nove principais categorias diagnósticas placentárias: placenta com morfologia normal, corioamnionite, vilite/ intervilosite, vasculopatia decidual (placenta com desordem circulatória materna), placenta com desordem circulatória fetal, atraso

na maturação vilositária, alterações sugestivas de aberrações genéticas, desordem de implantação e outras lesões (76).

A avaliação do acometimento placentário no curso da gestação é realizada através da US e parâmetros Dopplervelocimétricos, com avaliação da artéria umbilical, cerebral média e artérias uterinas, a fim de diagnosticar e monitorar uma suspeita de RCF e sinalizar as pacientes com maior risco de desenvolverem pré-eclâmpsia e parto prematuro. Além disso, a avaliação da estimativa de peso fetal quando abaixo da esperada aponta os casos que podem necessitar de um acompanhamento mais detalhado. Como as apresentações e complicações são bastante heterogêneas nas gestantes com doença falciforme, não há um protocolo definido para a frequência de realização da US pré-natal. No geral o seguimento é feito mensalmente a partir de 18 (39) ou 24 semanas (12) e quinzenalmente a partir do terceiro trimestre (39). Entretanto, cada caso deve ser acompanhado dependendo da sua evolução clínica e complicações apresentadas ao longo da gestação.

A anemia, independente da sua causa, cursa com aumento do risco materno e perinatal e, apesar de ser uma condição frequente necessita de avaliação antenatal mais detalhada, tratamento e seguimento individualizados de acordo com sua causa, a fim de reduzir o impacto negativo dessa condição na gestação.

2.OBJETIVOS

2.1 Objetivo Geral

Avaliar a presença da anemia e doença falciforme na gestação, seu impacto nos resultados maternos e perinatais e comparação dos dados entre os diferentes genótipos da doença falciforme.

2.2 Objetivos Específicos

1. Descrever a prevalência de anemia em gestações a termo e pré-termo e comparar com resultados maternos e perinatais;

2. Realizar revisão narrativa da literatura sobre resultados maternos e perinatais nas gestantes portadoras de doença falciforme e cuidados durante o pré-natal;

3. Comparar resultados maternos, perinatais e achados placentários nos diferentes genótipos de gestantes com doença falciforme acompanhadas em um centro de referência para alto risco.

3.MÉTODOS

3.1 Estudo 1

3.1.1 Desenho do Estudo

Análise secundária do Estudo Brasileiro Multicêntrico de Investigação de Prematuridade (EMIP) com o objetivo de avaliar a prevalência de anemia em partos de termo e pré-termo e entre as diferentes causas de prematuridade (trabalho de parto prematuro espontâneo, ruptura prematura de membranas ovulares, indicação médica de interrupção da gestação), correlacionando achados maternos e neonatais.

3.1.2 Constituição do EMIP

O EMIP foi um estudo de corte transversal prospectivo com um componente caso controle aninhado realizado em 20 hospitais de referência brasileiros localizados nas 3 regiões mais populosas do país. Todas as participantes do estudo assinaram o termo de Consentimento Livre e Esclarecido após serem explicadas e entendidas as condições do estudo (Anexo 1). O estudo foi aprovado pelo Comitê Nacional de Ética em Pesquisa (CONEP) e pelo comitê de ética de cada instituição participante (#0564.1.146.000-09) (Anexo 2).

Foram registrados 33740 nascimentos entre abril 2011 e julho 2012 nessas instituições participantes. Foi criado um banco de dados de todos os partos prematuros e seus neonatos para possibilitar classificação das causas de prematuridade (espontânea, secundária à ruptura prematura de membranas e indicação médica de interrupção da gestação). Além disso, foi implementada uma amostra de caso-controle para comparação dos partos pré-termo com amostra de partos a termo. Após cada parto pré-termo, foi incluído o parto a termo subsequente se a paciente concordasse em participar, até atingir o

número necessário estimado. O cálculo do tamanho amostral para os partos a termo foi baseado na prevalência de partos prematuros no Brasil em 6.5% em 2006, totalizando o mínimo de 1054 sujeitos em cada componente do estudo caso-controle (77, 78).

As informações foram coletadas em entrevista com a paciente na admissão para parto e em prontuários médicos até 60 dias após, caso o neonato tenha permanecido hospitalizado (79, 80).

3.1.3 Análise secundária

Para a análise secundária, as mulheres que não possuíam informações acerca de anemia e nível de hemoglobina foram excluídas. As demais foram divididas em 2 grupos, dependendo da presença ou não de anemia e a análise foi feita para o grupo pré-termo (antes de 37 semanas), termo (após 37 semanas) e comparando os 2 grupos entre si. Anemia foi definida OMS como hemoglobina abaixo de 11g/dL em qualquer fase da gestação (81).

Foi descrita a prevalência de anemia nos 2 grupos e entre as diferentes causas de prematuridade (espontânea, secundária à ruptura prematura de membranas e indicação médica de interrupção da gestação). Além disso, foram comparados os desfechos maternos e perinatais no grupo pré-termo com e sem anemia, no grupo termo com e sem anemia e ainda considerando apenas as pacientes com anemia, comparação entre os grupos pré-termo e termo.

As variáveis analisadas nesse estudo foram: características sócio demográficas (idade materna, área urbana ou rural, região do país (Sudeste, Nordeste, Sul), cor da pele, estado marital, anos de estudo, morbidade prévia, antecedentes obstétricos (outro filho com menos de 5 anos de idade, cesariana

prévia, intervalo entre as gestações, parto pré-termo prévio, antecedente de neonato abaixo de 2500g de peso de nascimento), adequação do cuidado pré-natal (início do pré-natal no 1º trimestre ou após, número de consultas de pré-natal, ganho de peso durante a gestação, índice de massa corpórea inicial, uso de álcool ou drogas ilícitas). Foi avaliado ainda, presença de infecção urinária, sangramento vaginal, presença de morbidade fetal, via de parto e desfechos neonatais (peso de nascimento, índice de Apgar no 5º. minuto <7, óbito fetal, necessidade de suporte ventilatório neonatal, morbidade neonatal, hemorragia intraventricular) e condição do neonato na alta médica.

Para variáveis categóricas tabelas de contingência foram usadas para testar possíveis associações. Para variáveis contínuas, foram descritas mediana, desvio padrão, valores mínimo e máximo. Análise estatística para variáveis categóricas foi realizada com teste Qui quadrado (X^2) ou exato de Fisher quando apropriado. Já para as variáveis contínuas, a análise foi realizada com teste Mann Whitney. Análise multivariada para avaliação de associação independente das variáveis com anemia tanto no termo como no pré-termo, foi realizada através de regressão logística. Considerado $p<0.05$ como estatisticamente significativo.

3.2 Estudo 2

3.2.1 Desenho do Estudo

Revisão narrativa da literatura. Os termos de busca utilizados na plataforma Pubmed, Scielo e Embase foram “sickle cell disease” e “pregnancy”, sendo selecionados os artigos na língua inglesa. A busca foi realizada desde a

criação das bases de dados até dia 09/04/2021, com foco na descrição de desfechos adversos e nas recomendações de cuidado durante o pré-natal.

Para descrição dos principais estudos, optou-se por compilar todas as revisões sistemáticas sobre o tema, descrevendo autoria, ano, número de sujeitos avaliados, tipo do estudo, desfechos encontrados e conclusão.

3.3 Estudo 3

3.3.1 Desenho do estudo

Estudo de coorte retrospectivo com revisão dos prontuários médicos e blocos de parafina coletados de pacientes acompanhadas no pré-natal especializado com doença falciforme de 2011 a maio 2017.

Para avaliação placentária, foram utilizados os laudos de avaliação anatomopatológica disponíveis no prontuário médico, além da avaliação morfológica de placentas de mulheres com doença falciforme, coletadas para projeto prévio do grupo de pesquisa do orientador do presente estudo (projeto: “Identificação de marcadores moleculares na etiologia da pré-eclâmpsia em pacientes portadoras de Doença Falciforme” - Com parecer CEP número 422.319 e CAEE 14092013.4.0000.5404 (Anexo 3).

3.3.2 Variáveis e Constantes

3.3.2.1 Constante

- **Doença falciforme**= genótipo da hemoglobina avaliado a partir de eletroforese de hemoglobina. Variável categórica: doença homozigótica ou anemia falciforme, quando presença de duas cadeias S de hemoglobina (HbSS); ou doença heterozigótica, quando combinação de uma cadeia S de hemoglobina com outra cadeia alterada da hemoglobina

não S, podendo ser hemoglobina C (HbSC), alteração na cadeia ß (HbSß talassemia);

3.3.2.2 Variáveis Independentes

- **Idade Gestacional (IG)** = número de semanas e dias da gestação em curso na ocasião da realização de US obstétrico ou de consulta pré-natal conforme informado em prontuário médico. Variável quantitativa, expressa em semanas e dias: desde a primeira consulta realizada no serviço até o parto.

3.3.2.3 Variáveis Dependentes

- **Avaliação morfológica da placenta:** avaliação de bloco de parafina, preparado para análise anatomopatológica, fixada, armazenada e avaliada quanto a maturidade, presença de fibrina no espaço interviloso, membrana sincicial, aglutinação nos vilos, nós sinciciais, hipoplasia de vilos distais, vilos avasculares, vilite crônica, excesso de fibrina perivilosa, dismorfismo vilositário- conforme definição detalhada no protocolo do Departamento de Patologia (76).
- **Complicações maternas:** intercorrências durante a gestação e especificadas em:

  - *Agravamento da anemia*: piora da anemia crônica já causada pela doença falciforme. Variável categórica: sim ou não.

  - *Infecção*: diagnóstico de qualquer infecção que complique a gestação. Variável categórica: sim ou não (82).

  - *Eventos trombóticos*: diagnóstico de trombose em qualquer parte do corpo, incluindo acidente vascular cerebral (AVC). Variável categórica: sim ou não.

- *Crise álgica*: episódios de dor aguda que pode acometer qualquer área do corpo, sendo mais frequente tórax, dorso, extremidades e abdome. Variável categórica: sim ou não (83).

- *Síndrome torácica aguda*: complicações pulmonares primárias de doença falciforme. Variável categórica: sim ou não (84).

- *morbidade materna grave ou near miss*, conforme critérios estabelecidos pela OMS (44)

- *óbito materno*: morte materna durante qualquer período da gestação, parto ou até 42 dias após o parto. Variável categórica: sim ou não.

- *Outros*: qualquer complicação não citada acima, incluindo pré-eclâmpsia, sequestro esplênico. Variável categórica: sim ou não.

- **Resultados Perinatais:** dados relacionados ao parto e coletados do prontuário, especificados em:

- *Peso de nascimento*: peso aferido ao nascimento em gramas. Variável quantitativa expressa em números inteiros 500 a 4000g.

- *Idade gestacional do parto*: anotado em semanas e dias baseado na data da última menstruação quando de certeza, ou em ultrassonografia realizada até 12 semanas de gestação, independente da viabilidade do concepto. Variável quantitativa, expressa em semanas e dias.

- *APGAR*: avaliação atribuída no 1º. e 5º. minuto de vida. Variável quantitativa expressa em números inteiros com valores de 0 a 10.

- *Desfecho na alta*: recém-nascido vivo na alta. Variável categórica: sim ou não.

- *Outras*: qualquer condição não explicitada acima, incluindo tempo de internação em UTI neonatal, cirurgia, Intubação Orotraqueal, droga vasoativa. Variável categórica: sim ou não.

- **Transfusão de sangue profilática:** se a gestante recebeu transfusão de sangue profilaticamente durante a gestação de acordo com o protocolo da instituição do estudo e não recebeu transfusão de sangue por complicações agudas. Variável categórica: sim ou não. Nos casos positivos, será anotado ainda a idade gestacional em que o procedimento foi realizado pela 1ª. vez e o intervalo entre as transfusões realizadas se mais de uma.
- **Transfusão de sangue terapêutica:** se a gestante recebeu transfusão de sangue sob demanda durante a gestação. Variável categórica: sim ou não. Nos casos positivos, será anotada ainda a idade gestacional em que o procedimento foi realizado pela 1ª. vez, o intervalo entre as transfusões realizadas se mais de uma e o nível de hemoglobina pré transfusional.

3.3.2.4 Variáveis de controle

- **Idade:** números de anos completos de vida obtido em prontuário no momento de início do seguimento, seja US ou consulta. Variável quantitativa expressa em números inteiros.
- **Cor ou Raça:** cor da pele da mulher, obtida pelo registro do prontuário. Variável categórica: branca, preta, parda, amarela, indígena ou outra.
- **Paridade:** número de vezes que a paciente engravidou, incluindo a gestação no momento da análise do prontuário. Variável quantitativa expressa em números inteiros: 1-8
- **Tipo de parto:** vaginal ou cesariana.

- **Trabalho de parto:** se o início do trabalho de parto foi espontâneo ou induzido
- **Antecedente obstétrico:** presença de comorbidades (descolamento de placenta, RCF, óbito fetal, óbito neonatal, abortamento) ou situações adversas nas gestações anteriores, independente da situação do concepto. Variável categórica: sim ou não.
- **Antecedentes pessoais:** antecedente de complicações clínicas anteriormente à gestação, incluindo: antecedente de acidente vascular cerebral (AVC), hipertensão arterial, Diabetes Mellitus, vasculopatia, nefropatia, uso de medicações. Variável categórica: sim ou não.

3.4 Seleção de sujeitos

Foram avaliados todos os prontuários médicos de pacientes que foram acompanhadas no Pré-Natal Especializado do Centro de Atenção Integral a Saúde da Mulher (CAISM) do Hospital Prof. Dr. Jose Aristodemo Pinotti por doença falciforme (confirmada com eletroforese de hemoglobina segundo registro em prontuário), de 2011 a 2017. Foram avaliados dados sociodemográficos, referentes às consultas de pré-natal, presença de intercorrências clínicas, procedimentos transfusionais, internações, dados referentes ao parto e ao recém-nascido, referidos no prontuário. Nos casos complicados ou que não seguem o curso esperado, foi avaliado o laudo anatomopatológico da placenta disponível no prontuário. Foram incluídas as pacientes que fizeram o seguimento no Pré-Natal Especializado no CAISM e tiveram seu parto na mesma instituição. Os casos em que o parto ocorreu em outra instituição foram excluídos da análise.

3.5 Procedimentos, Técnicas e Exames

A análise anatomopatológica placentária foi realizada nos blocos de parafina preparados para esse fim. Após a dequitação placentária no parto, a placenta foi colocada em formol, refrigerada e enviada para o setor de anatomia patológica, onde foram preparados os blocos de parafina com o material e a partir do bloco foram cortadas lâminas de 0,5mm para avaliação microscópica. Foram avaliadas quanto à maturidade, presença de fibrina no espaço interviloso, membrana sincicial, aglutinação nos vilos, nós sinciciais, hipoplasia de vilos distais, vilos avasculares, vilite crônica, excesso de fibrina perivilosa, dismorfismo vilositário- conforme definição detalhada no protocolo do Departamento de Patologia, seguindo a referência de Turowski G e cols (76).

3.6 Coleta de Dados

A coleta dos dados foi realizada pelo pesquisador responsável a partir da análise dos prontuários das pacientes e os dados relevantes foram transcritos para as fichas de coleta de dados específicas.

3.7 Processamento e Análise dos Dados

Todos os dados coletados foram inseridos em um Banco de Dados constituído para o estudo, no programa *Excell for Windows* e analisados com auxílio do *Epi Info* 7. Inicialmente foi feita uma análise descritiva, com prevalência dos diversos casos de doença falciforme (diferentes genótipos), resultados maternos, perinatais e também morfologia placentária. As variáveis contínuas foram apresentadas em média (M) e Desvio Padrão (DP) e as variáveis categóricas foram divididas em grupos e apresentadas em percentual (%) de frequência.

Para as variáveis contínuas, a normalidade da distribuição de dados foi avaliada pelo teste de Shapiro-Wilk, que se o valor de p for menor que p <0,05, a distribuição de dados foi considerada com distribuição não normal, de forma que os testes não paramétricos foram considerados para análise. No entanto, se p> 0,05, os dados foram considerados com distribuição normal e os testes paramétricos foram considerados para análise.

Para a comparação de duas variáveis contínuas com distribuição normal foi considerado o teste t de Student e para três ou mais variáveis contínuas com distribuição normal o teste ANOVA foi o teste estatístico escolhido. Para comparações de variáveis dicotômicas, foram considerados o Qui-quadrado ou o exato de Fisher.

As recomendações da Declaração STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) foram seguidas visando à padronização na apresentação dos resultados (85).

3.8. Aspectos Éticos

A análise secundária do EMIP seguiu os princípios éticos enunciados na Declaração de Helsinque, sobre pesquisa médica envolvendo seres humanos (86). Esteve de acordo com as diretrizes e normas regulamentadas na Resolução 196/96 do Conselho Nacional de Saúde sobre Pesquisas envolvendo seres humanos (87), vigente à época da coleta de dados. As pacientes que participaram do estudo concordaram com a participação no mesmo após entendimento e aceitação das condições e, assinaram um Termo de Consentimento Livre e Esclarecido (Anexo 1).

O Conselho Nacional de Ética em Pesquisa [CONEP, Ministério da Saúde do Brasil] e os Comitês de Ética em Pesquisa de cada instituição participante analisaram e aprovaram o estudo (Anexo 2).

O estudo sobre avaliação dos genótipos na doença falciforme não incluiu nenhum tipo de intervenção, apenas consulta de prontuário e revisão de blocos de parafina (de material placentário) quando necessário. As informações obtidas foram somente utilizadas para fins que são próprios da pesquisa, com a responsabilidade de manutenção do sigilo com relação à identidade dos casos incluídos na divulgação dos dados do estudo, conforme a resolução 466/12 do Conselho Nacional de Saúde.

Foi autorizada dispensa do Termo de Consentimento Livre e Esclarecido (TCLE) pelo Comitê de Ética em Pesquisa (CEP), uma vez que o estudo envolveu revisão de dados de prontuários e não houve intervenções junto às pacientes.

Os dados foram salvos em arquivos fechados e seguros, sob responsabilidade do pesquisador, durante o período de sessenta meses. Após este período, o arquivo será destruído.

4. RESULTADOS

Os resultados deste estudo serão apresentados em forma de artigos científicos. Cada artigo refere-se a um objetivo específico. As confirmações de submissão dos artigos encontram-se no Anexo 4.

Artigo 1

The role of anemia in term and preterm pregnancies: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP)

Camilla O. Figueira[1]; Helena M Gomide[1]; José P. Guida[1]; Tabata Z. Dias[1]; Giuliana J. Lajos[1], Ricardo P. Tedesco[2], Marcelo L. Nomura[1], Samira M. Haddad[1], Patrícia M. Rehder[1], Rodolfo C. Pacagnella[1], Maria H. Sousa[3]; José G. Cecatti[1,3]; Renato Passini Jr[1]; Fernanda G. Surita[1]; Maria Laura Costa[1,3]; Brazilian Multicenter Study on Preterm Birth study group*

Artigo 2

Main complications during pregnancy and recommendations for adequate antenatal care in Sickle cell disease: a review of literature

Camilla Olivares Figueira[1], Fernanda G. Surita[1], Kleber Y. Fertrin[2], Guilherme de Moraes Nobrega[1], Maria Laura Costa[1]

Artigo 3

High maternal perinatal morbidity and placental abnormalities in sickle cell disease pregnancies: a single-center retrospective analysis

Figueira CO[1], Guida JPS[1], Surita FG[1], Antolini A[2], Saad ST[3], Fertrin KY[4], Costa ML[1]

4.1 Artigo 1

The role of anemia in term and preterm pregnancies: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP)

Camilla O. Figueira[1]; Helena M Gomide[1]; José P. Guida[1]; Tabata Z. Dias[1]; Giuliana J. Lajos[1], Ricardo P. Tedesco[2], Marcelo L. Nomura[1], Samira M. Haddad[1], Patrícia M. Rehder[1], Rodolfo C. Pacagnella[1], Maria H. Sousa[3]; José G. Cecatti[1,3]; Renato Passini Jr[1]; Fernanda G. Surita[1]; Maria Laura Costa[1,3]; Brazilian Multicenter Study on Preterm Birth study group*

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Abstract

OBJECTIVE: To evaluate the prevalence of anemia in term and preterm pregnancies and compare maternal and perinatal outcomes among groups. **METHODS**: Secondary analysis of the Brazilian Multicenter Study on Preterm Birth (EMIP). A cross sectional study on preterm birth, with a nested case-control to enroll term pregnancies and enable the evaluation of risk factors and comparisons. The current study compared the prevalence of anemia in term and preterm pregnancies and among the different causes of prematurity (spontaneous preterm labor, preterm premature rupture of membranes (pPROM), or medically indicated preterm), correlating maternal and neonatal findings. This comparison was also performed considering only the women with anemia in both groups. Multivariate analysis to identify conditions that were independently associated to anemia in term and preterm groups was also performed. **RESULTS:** The study considered 3716 preterm and 1048 term births with available data on hemoglobin level. Overall prevalence of anemia was 31.70%, with 33.23% and 27.74% in preterm and term pregnancies respectively. Frequency of anemia was significantly higher among the pPROM group, present in 36.3% of the women (p=0.029). Less than 8 schooling years (p=0.003), maternal age <19 years old (p=0.047), non-white skin color (p<0.001) and having children under 5 years (p<0.001) were conditions associated with anemia and preterm birth. Also, in this group, anemia was associated with adverse perinatal outcomes, including need of ventilatory support (p=0.003), sepsis (p=0.006) and endocrine dysfunction (hypoglycemia) (p=0.001), small for gestational age newborns (p<0.001), 5-minute Apgar score <7 (p=0.001), and neonatal death (p=0.002). Multivariate analysis showed an association with living area and anemia in term group (p= 0.001), urinary tract infection and anemia in spontaneous prematurity and medically indicated group (p<0.001) and neonatal morbidity (p= 0.001) and inadequate number of prenatal care visits (p= 0.009) and anemia in pPROM group. **CONCLUSION**: Anemia was associated with poor maternal education, presence of children below 5 years old, late onset of prenatal care and less than six prenatal care visits. Also, it is more prevalent in preterm births, especially among cases of premature rupture of membranes and could be either associated to its cause or consequence, worsening an unfavorable condition.

KEYWORDS: anemia, pregnancy, preterm birth

Background

Prematurity is a major health problem and the main cause of neonatal morbidity and mortality (78, 80). World Health Organization (WHO) estimates an incidence of 15 million preterm deliveries worldwide. In Brazil, the incidence of prematurity is reported between 9.9% to 12.3% (78, 80), accounting for major health costs and burden. The main underlying causes of preterm delivery are spontaneous preterm labor, preterm premature rupture of membranes (pPROM) or medically indicated pregnancy interruption due to adverse maternal and/or fetal conditions (78, 80, 88), with estimated rates of 40-45%, 25-30% and 30-35% respectively (88).

Physiopathology of preterm birth is not fully understood and is described as multifactor, associated to social and environmental conditions that can be difficult to identify. It is estimated that around 50% of these births remain without a defined cause after childbirth (78, 88). Risk factors for prematurity include maternal malnutrition, short interval to a previous delivery (6 months or less), previous preterm birth, infections, smoking and anemia (78, 80, 88). Also, multiple pregnancy and conditions that can affect fetal well-being such as preeclampsia, placental dysfunction and fetal growth restriction are responsible for increasing prematurity rates in the group of medically indicated cases (81).

Anemia in pregnancy is defined based on WHO criteria as hemoglobin level below 11g/dL (5, 6). WHO estimates a prevalence of 41.8% anemia in pregnant women, but it varies in low- and high-income countries (5, 6, 80, 88). Several studies showed anemia as a risk factor for prematurity, pPROM, cesarean

delivery, low birth weight, high neonatal morbidity, maternal blood transfusion and infections, gestational hypertension and preeclampsia (5-8, 89, 90).

As prematurity is highly associated with neonatal morbidity and mortality and is also associated to the presence of anemia, this study, as a secondary analysis of a major multicentric trial, intends to evaluate the prevalence of anemia during preterm and term pregnancies and its association with poor maternal and perinatal outcomes.

Methods

The present study is a secondary analysis of Brazilian Multicenter Study on Preterm Birth (EMIP- Estudo Multicêntrico de Investigação de Prematuridade). The study was reviewed and approved by the National Council for Ethics in Research (CONEP) and by the Institutional Review Board of each participant center (#0564.1.146.000-09). All women included in the study signed an individual informed consent after understanding the conditions of the study.

The study protocol and complete methodology has been previously published (1, 11), however, in summary, the EMIP study was conducted in 20 referral obstetric hospitals in three Brazilian regions: Southeast, Northeast and South. It was a cross sectional study that evaluated 33740 births from April 2011 to July 2012, creating a database on preterm delivery and preterm infants. Then, causes of preterm delivery were separately described. Also, it assessed a nested unmatched case-control study, analyzing a term birth immediately after a preterm one.

For the main study, sample size was calculated based on Brazilian preterm birth prevalence at the time, reported as 6.5% in 2006 with allocation of at least 1054 subjects in each group for the case control analysis (78). Information was collected from an interview with the women during admission for childbirth and through medical records until 60 days if the neonate remained hospitalized (1,11).

For this secondary analysis, women with no information about anemia and hemoglobin levels, in their medical records were excluded. The remaining women were divided in two groups, according to the presence of anemia, and analysis performed for preterm (before 37 weeks), term (37 weeks or more) and comparing both conditions. Anemia was defined as hemoglobin level below 11g/dL anytime during pregnancy, according to WHO definition (81).

The prevalence of anemia was described in both term and preterm groups, and among different causes of prematurity (spontaneous preterm labor, preterm premature rupture of membranes or medically indicated pregnancy interruption). Also, we compared the findings of maternal and perinatal outcomes in the preterm group with and without anemia, in the term group with and without anemia and considering only the women with anemia, the comparison between term and preterm birth was also performed.

The variables analyzed for this study included sociodemographic characteristics (maternal age, urban or rural area, region of the country (Southeast, Northeast, South), skin color, marital status, years of study, previous morbidity, previous obstetric history (other children under 5 years old, previous cesarean section, interval between pregnancies, previous preterm birth, previous newborn under 2500g), adequacy of prenatal care (evaluated through onset of prenatal care at first trimester or later, number or prenatal care visits, gestational weight gain, initial body mass index, use of alcohol or drugs). We also analyzed presence of urinary tract infection, vaginal bleeding, presence of fetal morbidity), route of childbirth, and neonatal outcomes as birth weight, 5^{th} minute APGAR score <7, fetal death, neonatal ventilatory support, neonatal morbidity, Intraventricular Hemorrhage and neonate's condition at discharge.

For categorical variables, contingency tables were used to assess possible associations. For continuous measures, median, standard deviation, minimum and maximum values were described.

Statistical analysis for categorical variables was performed with Chi (X^2) Square test or Fisher's exact test where appropriate, while for continuous variables analysis was performed with Mann Whitney. Then, a multivariate analysis was staged to evaluate whether any variable/condition was independently

associated with anemia in term and preterm groups. For that, logistic regression was performed. We considered P-value of <0.05 as statistically significant. Data obtained were analyzed using SAS (Statistical Analysis System) for windows, version 9.4. SAS Institute Inc, 2002-2008, Cary, NC, USA.

Results

The main EMIP study identified 4150 preterm births (≤ 37 weeks gestation) during data collection and selected a sample of 1156 term births as a control group. For our secondary analysis we excluded 434 (10.46%) and 108 (9.42%) cases from preterm and term groups respectively, due to lack of information about the hemoglobin level. Among all preterm births, 1278 women (34.39%) had spontaneous preterm labor, 1066 (28.69%) preterm premature rupture of membranes (pPROM) and 1372 women (36.92%) needed a medically indicated premature delivery (Figure 1).

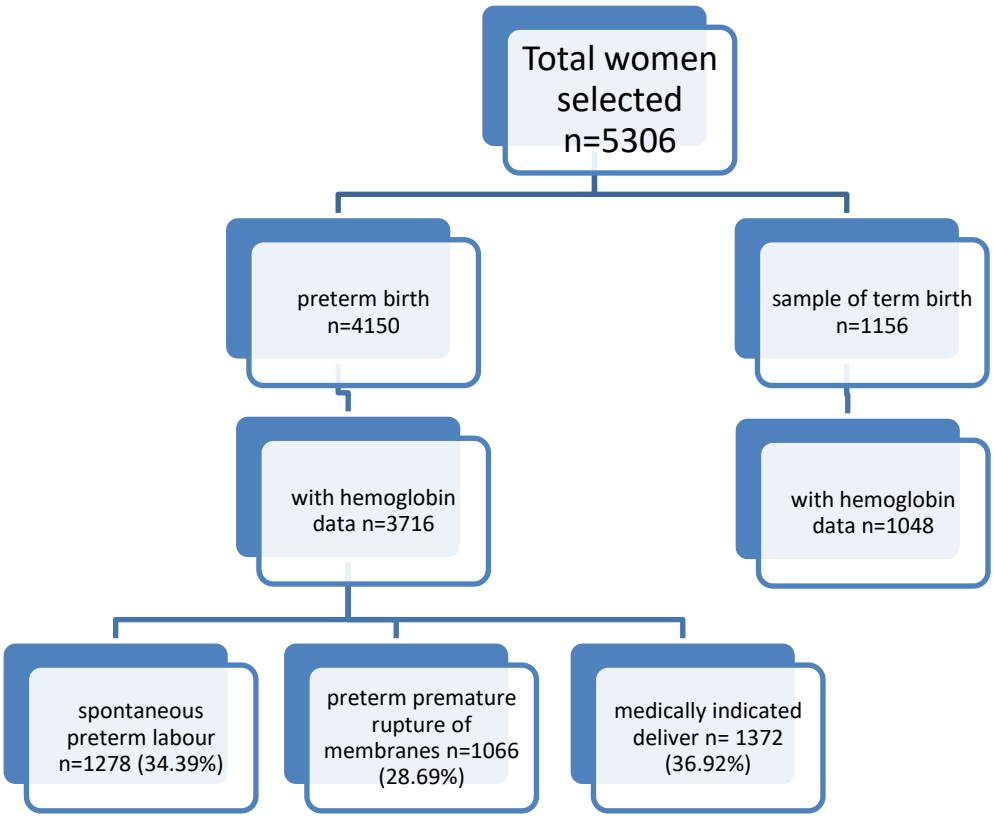

Figure 1. Flowchart of analyzed cases

The overall prevalence of anemia in preterm pregnancies was 33.23% and in term deliveries it was 27.74% (p=0.01). Frequency of anemia was significantly higher among the pPROM group present in 36.3% of the women (p=0.029). Among spontaneous preterm labor the frequency of anemia was 32.79% and in the medically indicated group, 31.27%.

Table 1 shows the maternal characteristics according to gestational age at birth and the presence of anemia. Age ≤19 years (p=0.047), non-white skin color (p=0.001), living in the Northeast region (p=0.001), ≤ 8 schooling years (p=0.003), and the presence of previous children under 5 years old (p=0.001), were significantly associated with anemia and preterm birth. Among the group of term childbirth, a statistically significant association with anemia was found among women living in urban area (p=0.001) and non-white skin color (p=0.015). Furthermore, comparing only the anemia cases, between preterm and term pregnancies, living in the Southeast region (p=0.026), ≤ 8 schooling years (p=0.001), ≤ 12 months

interpartal interval (p=0.021), previous preterm birth (p=0.001) and previous newborn with ≤2500g (p=0.001) were associated with preterm deliveries.

Table 1. Maternal profile according to age of birth and presence of anemia

Maternal profile	Gestational age at birth						
	< 37 weeks			≥ 37 weeks			p
	Anemia yes n (%)	Anemia no n (%)	p	Anemia yes n (%)	Anemia no n (%)	p	Anemia Yes <37 x ≥ 37
Maternal age (years)	1235	2481	0.047	288	750	0.559	0.292
≤ 19	276 (22.35)	472 (19.02)		58 (20.14)	130 (17.33)		
20-34	786 (63.64)	1626 (65.54)		197 (68.40)	535 (71.33)		
≥35	173 (14.01)	383 (15.44)		33 (11.46)	85 (11.33)		
Housing area	1231	2471	0.239	287	746	0.001	0.113
Rural	103 (8.37)	236 (9.55)		16 (5.57)	94 (12.60)		
Urban	1128 (91.63)	2235 (90.45)		271 (94.43)	652 (87.40)		
Region	1235	2481	0.001	288	750	0.65	0.026
Southeast	703 (56.92)	1388 (55.95)		143 (49.65)	392 (52.27)		
Northeast	399 (32.31)	725 (29.22)		117 (40.63)	296 (39.47)		
South	133 (10.77)	368 (14.83)		28 (9.72)	62 (8.27)		
Skin color	1235	2481	<0.001	288	750	0.015	0.064
White	493 (39.92)	1181 (47.60)		98 (34.03)	317 (42.27)		
Other	742 (60.08)	1300 (52.40)		190 (65.97)	433 (57.73)		
Marital status	1235	2481	0.067	288	750	0.817	0.081

With a partner	940 (76.11)	1954 (78.76)		233 (80.90)	602 (80.27)		
Without a partner	295 (23.89)	527 (21.24)		55 (19.10)	148 (19.73)		
Schooling (years)	1229	2433	0.003	287	737	0.257	0.001
≤ 8	530 (43.12)	912 (37.48)		91 (31.71)	274 (37.18)		
9-12	609 (49.55)	1305 (53.64)		172 (59.93)	408 (55.36)		
>12	90 (7.32)	216 (8.88)		24 (8.36)	55 (7.46)		
Morbid history	1125	2344	0.001	272	715	0.289	0.665
No	887 (78.84)	1829 (78.03)		216 (79.41)	589 (82.38)		
High blood pressure	71 (6.31)	171 (7.30)		12 (4.41)	33 (4.62)		
Diabetes	14 (1.24)	25 (1.07)		2 (0.74)	7 (0.98)		
Blood Disease	23 (2.04)	14 (0.60)		6 (2.21)	5 (0.70)		
Other	130 (11.56)	305 (13.01)		36 (13.24)	81 (11.33)		
Children under 5 years	1234	2479		288	749		
Yes	368 (29.82)	573 (23.11)	<0.001	85 (29.51)	193 (25.77)	0.222	0.981
No	866 (70.18)	1906 (79.89)		203 (70.49)	556 (74.23)		
Previous caesarean section	1234	2481		288	750		
Yes	276 (22.37)	546 (22.01)	0.803	66 (22.92)	153 (20.40)	0.373	0.840
No	958 (77.63)	1935 (77.99)		222 (77.08)	597 (79.60)		
Inter-pregnancy interval	732	1443		161	423		
≤ 12 months	74 (10.11)	124 (8.59)	0.245	7 (4.35)	24 (5.67)	0.523	0.021
>12 months	658 (89.89)	1319 (91.41)		154 (95.65)	399 (94.33)		
Previous PTB*	1230	2475		288	750		

Yes	242 (19.67)	473 (19.11)	0.682	23 (7.99)	64 (8.53)	0.775	<0.001
No	988 (80.33)	2002 (80.89)		265 (92.01)	686 (91.47)		
Previous newborn < 2500g	1224	470		288	743		
Yes	208 (16.99)	410 (16.60)	0.762	20 (6.94)	53 (7.13)	0.915	<0.001
No	1016 (83.01)	2060 (83.40)		268 (93.06)	690 (92.87)		

*PTB: preterm birth

Gestational characteristics associated with preterm birth and the presence of anemia includes late onset of prenatal care (p=0.001), <6 prenatal care visits (p=0.007), being pre-pregnancy underweight or normal weight (p=0.001), use of drugs before or during pregnancy (p=0.037), urinary tract infection (p=0.001) and vaginal bleeding (p=0.001), as shown in Table 2. Comparing only the anemia group, the absence of prenatal care (p=0.001), <6 prenatal care visits (p=0.001), Gestational weight gain ≤ 7 (p=0.001), urinary tract infection (p=0.005) and vaginal bleeding (p=0.001) were associated with prematurity. Also, cesarean section was more frequent in the preterm/anemia group (p=0.0026).

Table 2 – Gestational profile according to age of birth and presence of anemia

	Gestational age at birth						
	< 37 weeks			**≥ 37 weeks**			**p**
Gestational profile	**Anemia yes**	**Anemia no**	**p**	**Anemia yes**	**Anemia no**	**p**	**Anemia Yes <37 x ≥ 37**
	n (%)	**n (%)**		**n (%)**	**n (%)**		
Prenatal care	1235	2481	0.001	288	750	0.564	0.001
Yes	1190 (96.36)	2444 (98.51)		288 (100)	747 (99.60)		
No	45 (3.64)	37 (1.49)		0 (0)	3 (0.40)		
Onset of prenatal care	1009	2161	0.001	255	664	0.006	0.443
First trimester	620 (61.45)	1453 (67.24)		150 (58.82)	454 (68.37)		
Second/third trimester	389 (38.55)	708 (32.76)		105 (41.18)	210 (31.63)		
Adequacy of number of prenatal care visits*	1061	2265	0.007	273	700	0.052	<0.001

Adequate (≥ 6)	538 (50.71)	1291 (57.00)		199 (72.89)	551 (78.71)		
Inadequate (<6)	523 (49.29)	974 (43.00)		74 (27.11)	149 (21.29)		
Weight gain in pregnancy	1077	2221	0.495	263	768	0.577	<0.001
≤ 7 kg	367 (34.08)	713 (32.10)		53 (20.15)	148 (21.83)		
8-12 kg	363 (33.70)	759 (34.17)		91 (64.60)	211 (31.12)		
>12 kg	347 (32.22)	749 (33.72)		119 (45.25)	319 (47.05)		
Initial body mass index	1090	2170	<0.001	261	666	0.352	0.982
<18,5 kg/m^2: underweight	111 (10.18)	156 (7.19)		25 (9.58)	50 (7.51)		
18,5–24,99 kg/m^2: normal weight	631 (57.89)	1160 (53.46)		152 (58.24)	372 (55.86)		
25-29,99 kg/m^2: overweight	222 (20.37)	501 (23.09)		55 (21.07)	144 (21.62)		
≥ 30 kg/m^2: obesity	126 (11.56)	353 (16.27)		29 (11.11)	100 (15.02)		
Alcohol drinking	1226	2464	0.576	286	745	0.141	0.176
Yes (often)	16 (1.31)	27 (1.10)		7 (2.45)	8 (1.07)		
No or few times	1210 (98.69)	2437 (98.90)		279 (97.55)	737 (98.93)		
Use of drugs	1235	2481	0.037	288	75	0.204	0.179
Never used	1162 (94.09)	2379 (95.89)		274 (95.14)	730 (97.33)		
Used before pregnancy	38 (3.08)	59 (2.38)		11 (3.82)	16 (2.13)		
Used during pregnancy	35 (2.83)	43 (1.73)		3 (1.04)	4 (0.53)		
Urinary tract infection	1223	2468	<0.001	288	748	0.614	0.005
Yes	560 (45.79)	895 (36.26)		106 (36.81)	288 (38.50)		
No	663 (54.21)	1573 (63.74)		182 (63.19)	460 (61.50)		
Anemia (patient report)	1206	2444	<0.001	286	746	<0.001	0.195

Yes	683 (56.63)	362 (14.81)		112 (39.16)	138 (18.50)		
No	523 (43.37)	2082 (85.19)		174 (60.84)	608 (81.50)		
Hb value	88	334	<0.001	22	92		0.199
>9,0 g/dL	79 (89.77)	334 (100)		22 (19.30)	92 (80.70)		
<9,0 g/dL	9 (10.23)	0 (0)					
Vaginal bleeding	1057	2067	<0.001	244	621	0.071	<0.001
Yes	185 (17.50)	248 (12.0)		9 (3.69)	43 (6.92)		
No	872 (82.50)	1819 (88.0)		235 (96.31)	578 (93.08)		
Fetal morbidity	1151	2312	0.222	267	701	0.523	<0.001
Malformation	56 (4,87)	150 (6.49)		3 (1.12)	9 (1.28)		
Fetal growth restriction	114 (9.90)	243 (10.51)		3 (1.12)	19 (2.71)		
Other	93 (8.08)	171 (7.40)		13 (4.87)	33 (4.71)		
No	888 (77.15)	1748 (75.61)		248 (92.88)	640 (91.30)		
Delivery route	1235	2481	0.238	288	750	0.802	0.002
Vaginal	518 (41.94)	1107 (44.62)		147 (51.04)	393 (52.40)		
Forceps/vacuum	19 (0.51)	26 (0.70)		10 (3.47)	34 (4.53)		
Cesarean	683 (55.30)	1312 (52.81)		128 (44.44)	314 (41.87)		
Vaginal + cesarean	15 (1.21)	36 (1.45)		3 (1.04)	9 (1.20)		

* Ministry of Health criteria

Table 3 shows perinatal outcomes according to gestational age at birth and the presence of anemia. In the preterm group, the presence of anemia was significantly associated with the need of ventilatory support (p=0.003), sepsis (p=0.006) and endocrine dysfunction (hypoglycemia) (p=0.001). This last one was the only association found between term delivery and anemia (p=0.012). In the analysis of the anemia group, newborns classified as small for gestational age were more frequent in the premature (25.41%) than term (1.74%) deliveries. Furthermore, association of anemia and prematurity was found for: 5-minute apgar score <7 (p=0.001), fetal death (p=0.002), endotracheal intubation at birth (p=0.001), use

of surfactant (p=0.001), ventilatory support (p=0.001), sepsis (p=0.001), respiratory distress (p=0.001), intraventricular hemorrhage (p=0.001), pneumonia (p=0.027), neonatal death (p=0.001).

Table 3 – Neonatal outcomes according to age at birth and presence of anemia

Neonatal Outcomes	**Gestational age at birth**						
	< 37 weeks			**≥ 37 weeks**			**p**
	Anemia yes n (%)	**Anemia no** n (%)	**p**	**Anemia yes** n (%)	**Anemia no** n (%)	p	**p anemia yes <37x≥ 37 weeks**
Birth weight	1232	2467	0.108	288	750	0.076	<0.001
≤1500 g	243 (19.72)	524 (21.24)		0 (0)	1 (0.13)		
1501 to 2500 g	656 (53.25)	1223 (49.57)		10 (3.47)	50 (6.67)		
>2500 g	333 (27.03)	720 (29.19)		278 (96.53)	699 (93.20)		
Adequacy of weight for gestational age	1232	2467	0.268	288	750	0.249	<.0001
Small	313 (25.41)	656 (26.59)		5 (1.74)	27 (3.60)		
Adequate	906 (73.54)	1771 (71.79)		232 (80.56)	604 (80.53)		
Large	13 (1.06)	40 (1.62)		51 (17.71)	119 (15.87)		
Apgar score 5º min <7	1227	2439	0.768	287	743	0.194	<.0001
Yes	116 (9.45)	238 (9.76)		0 (0.00)	6 (0.81)		
No	1111 (90.55)	2201 (90.24)		287 (100)	737 (99.19)		
Fetal death	1235	2481	0.232	288	750		0.002
Yes	40	100		0 (0)	0 (0)		

	(3.24)	(4.03)					
No	1195	2381		288	750		
	(96.76)	(95.97)		(27.75)	(72.25)		
Endotracheal intubation at birth	1181	2338	0.919	270	678	0.946	<.0001
Yes	191	375		5	13		
	(16.17)	(16.04)		(1.85)	(1.92)		
No	990	1963		265	665		
	(83.83)	(83.96)		(98.15)	(98.08)		
Surfactant use	1159	2307	0.637	262	671	0.483	<.0001
Yes	186	356		1	1		
	(16.05)	(15.43)		(0.38)	(0.15)		
No	973	1951		261	670		
	(83.95)	(84.57)		(99.62)	(99.85)		
Fetal Malformation	1166	2315	0.942	265	674	0.361	0.0002
Yes	128	256		9	32		
	(10.98)	(11.06)		(3.40)	(4.75)		
No	1038	2059		256	642		
	(89.02)	(88.94)		(96.60)	(95.25)		
Ventilatory support	1178	2341	**0.003**	268	680	0.348	<.0001
Yes	645	1158		19	61		
	(54.75)	(49.47)		(7.09)	(8.97)		
No	533	1183		249	619		
	(45.25)	(50.53)		(92.91)	(91.03)		
Any neonatal morbidity	1181	2336	**0.001**	268	682	0.874	<.0001
Yes	876	1585		61	152		
	(74.17)	(67.85)		(22.76)	(22.29)		
No	305	751		207	530		
	(25.83)	(32.15)		(77.24)	(77.71)		

Sepsis	839	1518	**0.006**	60	149	0.161	<.0001
Yes	273 (32.54)	413 (27.21)		2 (3.33)	14 (9.40)		
No	566 (67.46)	1105 (72.79)		58 (96.67)	135 (90.60)		
Respiratory distress	865	1572	0.457	62	149	0.448	<.0001
Yes	661 (76.42)	1180 (75.06)		31 (50.00)	66 (44.30)		
No	204 (23.58)	392 (24.94)		31 (50.00)	83 (55.70)		
Pulmonary air leak	827	1483	0.767	60	146	1.00	0.162
Yes	35 (4.23)	59 (3.98)		0 (0.00)	2 (1.37)		
No	792 (95.77)	1424 (96.02)		60 (100)	144 (98.63)		
Intraventricular Hemorrhage	688	1192	0.867	53	109		**0.010**
Yes	68 (9.88)	115 (9.65)					
No	620 (90.12)	1077 (90.35)		53 (32.72)	109 (67.28)		
Endocrine dysfunction	857	1539	**0.001**	62	146	**0.012**	0.166
Yes	235 (27.42)	335 (21.77)		12 (19.35)	11 (7.53)		
No	622 (72.58)	1204 (78.23)		50 (80.65)	135 (92.47)		
Necrotizing enterocolitis	852	1535	0.141	62	146		0.251
Yes	28 (3.29)	35 (2.2)		0 (0)	1 (0.68)		
No	824 (96.71)	1500 (97.72)		62 (10%)	145 (99.32)		
Pneumonia	852	1543	0.388	62	146	1.000	**0.027**

Yes	59	93		0	2	
	(6.92)	(6.03)		(0.)	(1.3)	
No	793	1450		62	144	
	(93.08)	(93.97)		(100)	(98.63)	
Oxygentherapy at 28days	851	1541	0.729	62	145	**0.013**
Yes	77	133		0	1	
	(9.05)	(8.63)		(0.00)	(0.69)	
No	774	1408		62	144	
	(90.95)	(91.37)		(100)	(99.31)	

In order to understand what conditions were independently associated to the occurrence of anemia in term and preterm pregnancies, we performed a multivariate analysis (Table 4). For the term group, housing area was the only statistically significant association. For the preterm group, the factors associated to anemia and preterm birth are listed in Table 4, and include: urinary tract infection in spontaneous prematurity and medically indicated groups (p<0.001) and neonatal morbidity (p= 0.001) and adequacy of prenatal care visits (p= 0.009) and anemia in pPROM group.

Table 4: Factors independently associated with anemia among term (model 1) or different preterm causes (model 2: spontaneous preterm labor), model 3: (medically indicated), model 4: (pPROM), in a multivariate analysis.

Model/ Variable	**OR**	**IC 95% p/ RP**	**p**
(a)Model 1 Term pregnancies [n=594]			
Housing area-urban	2.71	1.12 to 6.54	**0.02**
(b)Model 2 Preterm- Espontaneous preterm labor (<37 weeks) [n= 735]			
- Skin color- non-white	1.50	1.10 to 2.05	**0.007**
- UTI	1.48	1.09 to 2.03	**0.01**
(c)Model 3, Preterm- Medically indicated [n=745]			
- UTI	2.19	1.57 to 3.04	**<0.01**
- Vaginal bleeding	2.19	1.39 to 3.44	**<0.01**
(d)Model 4, Preterm- pPROM [n=610]			
- any neonatal morbidity	1.66	1.13 to 2.42	**0.001**

- Inadequate number of prenatal care visits	1.52	1.08 to 2.15	**0.009**
- children under 5 years old	1.48	1.00 to 2.20	**0.048**

UTI: urinary tract infection; pPROM: preterm premature rupture of membranes. *Independent variables analyzed*: housing area, maternal age, region of the country, skin color, morbid history, children under 5 years, prenatal care, onset of prenatal care, adequacy of number of prenatal care visits, initial body mass index, use of drugs, urinary tract infection, vaginal bleeding, neonatal ventilator support, any neonatal morbidity.

Discussion

The overall prevalence of anemia in our study was 31.70%, with 27.74% in term deliveries and 33.23% among preterm deliveries. Anemia is a public health problem worldwide, especially in low and middle-income countries (79, 91, 92) and it is associated with worse maternal and perinatal outcomes, including increased maternal and perinatal death, preterm birth, cesarean section, preeclampsia and low birth weight (93, 94). WHO National estimates report anemia prevalence of 41.8% (81), higher than our results.

Furthermore, our study presented higher prevalence of anemia then reported in a study in Lagos, Nigeria in 2020, that was 20.0% (95), higher than a multicenter Chinese study that found an overall prevalence of anemia of 23.5% (96) higher than a study conducted in Jerusalem with 10.5% of anemia (97) and higher than a large retrospective study in British Columbia that found anemia in 12.8% of its records (98). The difference in findings throughout studies may represent the population analyzed as anemia is more prevalent in low-income countries and in regions with poor antenatal care (99). A Brazilian study with adolescents showed an overall prevalence of anemia of 41% (10). This higher prevalence may be due to the additional risk factors related to pregnancy in adolescents as higher need of iron intake (100) and nutritional deficiencies (101). Also, our study was conducted in referral obstetric hospitals in the three most populous Brazilian regions, so the prevalence of anemia could have been higher as the most challenging and severe cases are treated in these hospitals.

Our results also found association with maternal age and the presence of anemia in preterm deliveries, as it was more frequent in pregnant adolescents. This is in agreement with the Chinese study that found higher prevalence of anemia in women under 20 (17). In addition, positive association for preterm birth and anemia was evidenced in non-white women, living in the Northeast region, schooling years less than 8, presence of children below 5 years old, onset of prenatal care at second or third trimester and less than six prenatal care visits. These findings are consistent with literature as anemia is associated with poor access to antenatal care and low socio-economic condition (6, 95, 96). In our country, non-white skin color and the Northeast region are associated with lower income regions and therefore associated with anemia (9).

Late onset of prenatal care was a finding associated with anemia in both term and preterm deliveries. It may be related to the fact that iron supplementation wasn´t long enough to recover at least the expected dilutional anemia and a chronic history of malnutrition and self-care. As a universal recommendation, prophylactic iron should be prescribed during gestation throughout postpartum (81).

Anemic women had high cesarean section rates in our study (especially among preterm deliveries) and such procedure can worsen hemoglobin level leading to other complications. Also, in the preterm group, fetal growth restriction was more frequent, and anemia could be partially responsible for the restriction. Previous preterm birth and previous low birth weight newborn were also more frequent in the preterm group. Moreover, anemia increases the risk for postpartum hemorrhage, worsening an already deficient hemoglobin level (7).

Considering factors independently associated to anemia, living in urban area was associated to anemia in term pregnancies. This could most likely be a consequence of the great majority of the population being urban, but also could be worsen by nutritional habits in urban centers where availability of food with low nutritional quality is higher. Differently, a study in China found that rural population is at greater risk for anemia (96), however studies correlate anemia with family income and schooling years, being poor education and low economic conditions associated to anemia (6, 96, 102). Urinary tract infection was associated with anemia in spontaneous preterm labor and medically indicated premature delivery. It might have been the trigger of spontaneous preterm labor and could deteriorate maternal clinical condition, as it is an already known risk factor for prematurity, independent the gestational age of the infection (103). In the spontaneous preterm labor, non-white skin color was also associated with prematurity, probably due to less assessment to health care. Having other child below 5 years old, any neonatal morbidity and inadequate numbers of antenatal care visits were associated with anemia and pPROM, suggesting that antenatal care is quite important to follow the women and identify alterations that can lead to complications. Studies suggest screening for infections, nutritional adequacy, iron supplementation and a periodic antenatal visit to check maternal health and early detection of possible pathologic conditions (98, 104).

Prematurity is enhancing worldwide and is a complex situation as the goals in order to reduce its rates are difficult to reach (105, 106). WHO estimates almost 15 million preterm deliveries every year (11.1% of all births) increasing costs in public and private healthcare (81). In our study, prematurity was divided into three etiologies: premature rupture of membranes, spontaneous premature labor and medically indicated preterm birth. The frequency of anemia in each group was 36.3%, 32.79% and 31.27% respectively, and in general, in the anemic patients, preterm birth was more frequent than delivery after 37 weeks of gestation, what is in agreement with the study in India (6) and the retrospective cohort studied in British Columbia that found an Odds Ratio of 2.44 for preterm birth and unspecified severity anemia (98). In all the three situations, anemia might be related to the cause of prematurity, but most likely to its consequence as time for iron supplementation was reduced.

Besides, prematurity costs millions to healthcare and is strongly associated with neonatal morbidity and mortality (98, 107, 108) with rates that have not decreased despite the knowledge and efforts to reduce its prevalence (106). The improvement of maternal and neonatal intensive care is a situation that tends to persist and enables women with several morbidities to control the underlying condition and pursue pregnancy and neonates delivered in a very preterm gestational age to survive, sustaining high rates of preterm birth.

The main study (EMIP) was a multicenter cross-sectional study with a nested case-control component and was conducted in 20 referral obstetric hospitals and in the largest Brazilian study in prematurity so far (1). One of its findings was that anemia is a risk factor for prematurity, what was an important point for further analysis.

It is important to point out that this study has limitations as it was based on a secondary analysis of a major multicentric study. As a consequence, some variables were difficult to standardize and it was hard sometimes to define the cases based on past medical records. Although it highlighted the high prevalence of anemia and prematurity in our setting and could bring light to further studies considering the association of anemia and preterm birth and the importance of additional studies to evaluate the topic and improve maternal healthcare aiming the reduction in neonatal morbidity and mortality rates caused by prematurity.

Acknowledgements:

Brazilian Multicenter Study on Preterm Birth study group:

Sergio T Marba, Ruth Guinsburg, Francisco E Martinez, Vilma Zotarelli, Lucio T Gurgel, Francisco E Feitosa, George N Chaves, Ana M Porto, Isabela C Coutinho, Antonio C Barbosa Lima, Elias F Melo Jr, Débora F Leite, Melania M Amorim, Adriana S O Melo, Fabiana O Melo, Marília G Martins, Marynea V

Nunes, Cláudio S Paiva, Moises D Lima, Djacyr M Freire, Edson G Tristão, Denis J Nascimento, Carlos A Menezes, Marcelo Aquino, Janete Vettorazzi, Cintia E Senger, Augusta M B Assumpção, Marcela A F Guedes, Maria E L Moreira, Vera T Borges, Nelson L Maia Filho, Jacinta P Mathias, Eduardo Souza, Ana C P Zamarian, Silvana M Quintana, Patrícia P S Melli, Fátima A Lotufo, Kaliane Uzilin, Elvira A Zanette, Carla B Andreucci, Tenilson A Oliveira, Laércio R Oliveira, Marcos A N Santos, Nelson Sass, Mirian R F Silveira, Pedro R Coutinho, Luciana Siqueira

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4.2 ARTIGO 2

Main complications during pregnancy and recommendations for adequate antenatal care in Sickle cell disease: a review of literature

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Abstract

Introduction: Sickle cell disease (SCD) is the most common monogenic disease worldwide with variable prevalence in each continent. A single nucleotide substitution leads to an amino acid change in the β globin chain, turning normal hemoglobin into a structurally abnormal one called hemoglobin S inherited in homozygosity (HbSS) or double heterozygosity (HbSC, HbSβ) that leads to chronic hemolysis, vaso-occlusion, inflammation, and endothelium activation. Pregnant women with SCD are at higher risk of maternal and fetal complications. **Methods:** Narrative review of literature considering Sickle Cell Disease and pregnancy, main clinical and obstetrical complications, specific antenatal care and follow-up for maternal and fetal surveillance. **Results:** Pregnant women with SCD are at higher risk of clinical and obstetric complications such as pain crises, worsening anemia, pulmonary complications, infections, thromboembolic events, preeclampsia, cesarean section, maternal death. They are also at increased risk of neonatal complications such as low birth weight, increased mortality, premature birth, stillbirth. Severe complications can occur in patients of any genotype. **Conclusion:** SCD is a high-risk condition that increases maternal morbidity and mortality. A multidisciplinary approach during pregnancy and postpartum is key to adequately diagnose and treat complications.

Keywords: sickle cell anemia, maternal morbidity, maternal mortality, pregnancy, antenatal care

Introduction

Sickle cell disease (SCD) is the most common monogenic recessive inherited disease worldwide and it has been first described over a century ago (11). Approximately 300,000 children are born with the disease every year (12-14) and the prevalence of the mutated gene varies in each continent. In America, the prevalence is about 0.49 in 1000 live births, 0.07 in Europe, 0.68 in South and Southern Asia, and 10.68 in Africa (15). In Brazil, there are 2 million people carrying the sickle cell gene mutation and about 25,000 to 50,000 people with the homozygous form of the disease (16). Incidence in Brazil varies according to the state from 1:650 live births in Bahia to 1:13,500 live births in Rio Grande do Sul (109).

A single nucleotide substitution leading to switching a glutamic acid residue to a valine one in the β globin chain turns normal hemoglobin into a structurally abnormal one called hemoglobin S (HbS) that causes SCD and is associated to endothelium activation and chronic inflammation. Other genotypes include hemoglobinopathy SC (HbSC) when the mutated S hemoglobin is associated with hemoglobin C, sickle beta thalassemia when hemoglobin S is associated with thalassemia mutations in the β chain (HbSβ) (11, 12, 17, 22). Those heterozygous forms may have better outcomes (18, 19), although they are also associated with higher morbidity and mortality, especially during pregnancy and postpartum.

Complications in pregnancy include higher frequency of hypertensive disorders, (including preeclampsia), thromboembolic events, fetal demise, fetal growth restriction, preterm birth (110) and higher risk of maternal death (19, 111). Also, sickle cell complications as pain episodes, acute chest syndrome, anemia and infections (38, 42) are common in pregnant women (110) and responsible for recurrent hospitalizations and morbidity.

Pathophysiology of sickle cell disease

Polymerization of deoxyhemoglobin S upon exposure to low oxygen levels deforms the membrane of red blood cells, which become elongated in the typical

sickled shape, more adherent to endothelial cells, and less flexible (22). The same changes can also occur in reticulocytes (21). Red cell sickling shortens their lifespan by being removed from circulation either by reticuloendothelial system or intravascular hemolysis (22). The release of free hemoglobin from within red cells along with sickling and cell adhesion to endothelial cells leads to endothelial activation, generates cytokines, and activates the coagulation cascade in a systemic inflammatory response (23). Neutrophil and platelet are also activated ultimately culminating in sickle cell vaso-occlusion (21, 22), a hallmark of the disease, which is responsible for acute complications as well as end organ damage due to ischemia and inflammation (22).

In the past 30 years, major advances in research have resulted in a better understanding of the pathophysiology of SCD and earlier interventions during childhood, including e.g., the use of penicillin, vaccinations, screening and prevention of stroke, which have improved patients' quality of life and have extended life expectancy of people with SCD allowing women to reach childbearing age (31-34) and pursue pregnancy.

SCD is a chronic inflammatory condition, and pregnancy in such women is considered a high-risk condition (27, 35-39) that needs a close follow up in specialized medical services with a multidisciplinary approach.

Clinical and Obstetric Complications in SCD

Several studies reported maternal complications of SCD including vaso-occlusive crises (VOCs, the most frequent cause of morbidity and hospitalization), infections (especially urinary tract), thromboembolic events (including deep venous thrombosis and stroke), pulmonary complications (the main cause of mortality), chronic renal failure, caesarian section, and maternal death (11, 38, 42). Fetal complications include fetal growth restriction (FGR), low birth weight (LBW), prematurity, fetal distress during labor and increased perinatal mortality (18, 26, 27, 38, 42). However, there is a wide range among published reports regarding the incidence of complications due to different study designs, country or world region of the studied cases, access to healthcare and

even the absence of statistical analysis to account for confounding variables (110).

A metanalysis in 2015 compared maternal and perinatal outcomes in women with and without SCD. The study analyzed 26,349 women with SCD and compared them in groups according to the SCD genotype (1,276 women with HbSS and 279 women with HbSC); however, the majority of SCD cases had no known genotype (24,794). The study reported an almost 18-fold increase in maternal mortality in women with SCD compared with non-SCD women, and a more than 2-fold risk for preeclampsia. No additional risk for eclampsia was found, except for those women with HbSS genotype (an almost 5-fold risk). They also reported a small increase in the risk of caesarean section in all SCD groups and a higher risk of stillbirth in both studied genotypes. Neonatal death and premature birth were twofold higher in the HbSS group only. Small for gestational age babies were more prevalent in SCD women than in the control group (18).

A study from 2010 about mortality in SCD and the use of hydroxyurea (the first approved drug to treat SCD by increasing fetal hemoglobin production) showed a reduction in SCD complications such as painful crisis, blood transfusion, and acute chest syndrome, increasing the chance of patient survival from 65 to 86% in the group not treated and treated with hydroxyurea, respectively (26). Although hydroxyurea is not approved for use in pregnancy, its use may be considered after pregnancy and breastfeeding in order to improve quality of life and reduce hospitalizations.

Another study in 2018 evaluated adverse outcomes in different SCD genotypes (HbSS, HbSC, and HbSβ thalassemia) included 89 women and found that 52% were hospitalized during pregnancy for clinical or obstetrical complications. The main reasons for hospitalization were VOC (41%) and obstetric adverse events (22%), and most of them occurred in HbSS patients. However, they did not find statistically significant differences among the SCD genotypes. Perinatal outcomes such as low birth weight (LBW), prematurity, preeclampsia, and stillbirth were more frequent in HbSS and HbSC groups, with no significant difference between the SCD groups. Postpartum adverse outcomes (hemorrhage, infections, and thromboembolic events) were

significantly more frequent among HbSβ thalassemia group (57%), compared with HbSS (18%) and HbSC (13%) groups (112).

An additional study with 62 SCD pregnancies compared the complications within the three genotypes (HbSS, HbSC, and HbSβ thalassemia). Urinary tract infection was the most common complication, with similar frequencies in HbSS and HbSC groups (30% and 33%, respectively). VOC was the second most prevalent complication and was more frequent among HbSC pregnancies (27%). Preeclampsia occurred in 11% of HbSS cases, 40% of HbSβ and 20% of HbSC pregnancies. Caesarean section was the delivery mode in 37%, 70%, and 40% of cases in HbSS, HbSC and HbSβ thalassemia groups, respectively. Prematurity rates were 41% for HbSS group and 23% for HbSC group, with no reported cases for HbSβ thalassemia. Stillbirth occurred only among HbSS group (11%) and no maternal deaths were reported in the study (113).

In Nigeria, a study compared 50 HbSS women with normal controls and the results showed a higher frequency of pregnancy-induced hypertension (28% in HbSS group and 6% in control group) and 32% of VOC in the SCD group. They also found 16% of patients with fetal growth restriction and no cases in controls. Preterm delivery was also more frequent in HbSS group (28% versus 10%). Overall, complications were significantly higher in the HbSS group, occurring in 92% women (versus 38% in controls) (36).

The high frequency of complications in SCD pregnancies corroborates the need for early diagnosis and surveillance to reduce morbidity and mortality. Maternal mortality ranges from 1% in a retrospective study in United Kingdom (25) to 9.2% in a Nigerian study (43). That discrepancy may be due to differences in quality of care, including early diagnosis and treatment of complications, as well as sub notification of cases.

Near miss is a condition in which women survive a severe complication in pregnancy, childbirth, or within 42 days postpartum. In Latin America, it is estimated there are 34 near miss cases to 1,000 live births (44) and 15 near miss cases to each maternal death (46, 47). In SCD pregnant women, about a third of them face a near miss event during pregnancy or puerperal cycle, especially due

to acute chest syndrome, a severe form of VOC affecting the lungs, and the leading cause of death in adult SCD patients (26, 48). Unsurprisingly, SCD increases the possibility of a woman suffering a near miss event during pregnancy and/or postpartum. Despite the acute maternal severity of a near miss condition, this event is also associated with LBW and very low birth weight newborns, newborn admission to intensive care, stillbirth, early neonatal death, and long maternal hospital stays (44, 49).

Studies with SCD in pregnancy usually involve a small number of patients due to the relative rarity of the condition and the difficulties in the compilation of data. A summary of metanalyses found in Pubmed, Scielo, and Embase about SCD during pregnancy is presented in Table 1.

Table 1. Systematic Reviews about Sickle Cell Disease during pregnancy

Author/year (country)	Study design	Number of studies and/or women	Outcomes	Conclusions
Oteng-Ntim et al., 2015 (London)	Meta-analysis	26,349 SCD women	MM (RR 18.51 95%CI, 8.63-39.72) PE (RR 2.06 95% CI 1.49-2.85) CS (RR, 1.27 95% CI, 1.18-1.36) ND (RR, 2.68 95%CI, 1.49-4.82)	Pregnant women with SCD have high risks or maternal and perinatal adverse outcomes Risks are greatest for those in low-income countries and for those with HbSS disease compared to HbSC
Boafor et al., 2015 (Ghana)	Meta-analysis	- 9 studies - 12 studies - 13 studies - 6 studies - 10 studies - 6 studies - 11 studies - 9 studies - 10 studies - 6 studies	MM (OR 10.91 95%CI 1.83-65.11, p=0.009) PE (OR 2.05 95% CI 1.47-2.85, p<0.001) CS (OR 1.54 95% CI 1.27-1.87, p<0.001) ND (OR 2.71, 95% CI 1.41-5.22, p<0.003) FGR (OR 2.69, 95% CI 1.85-4.21 p<0.001) Perinatal mortality (OR 3.76, 95% CI 2.34-6.06, p<0.001) Prematurity (OR 2.14, 95% CI 1.56-2.95, p<0.001) LBW (OR 2.00 95% CI 1.42-2.83, p<0.001) Stillbirth (OR 4.05 95% CI 2.59-6.32, p<0.001) Infection (OR 2.48 95% CI 1.23-5.01, p= 0.011)	SCD increases the risk of maternal and perinatal outcomes in low- and high-income countries
Inparaj et al., 2020 (London)	Meta-analysis	3,964 Patients	- ACS/ pneumonia (event rate 6.46%, 95% CI 4.66-8.25) - Pulmonary thromboembolism (RR 7.74, 95% CI 4.65-12,89)	Strong association between SCD and maternal pulmonary complications

MM: Maternal mortality, PE: Preeclampsia, CS: Cesarian section, ND: Neonatal Death, FGR: Fetal growth restriction, LBW: Low birthweight
ACS: acute chest syndrome

Antenatal Care

As a chronic systemic condition, pregnancy in SCD should ideally be planned in order to minimize possible complications. One main concern is over the use of medications for SCD during pregnancy and lactation. Common medications used in SCD management that should be discontinued before and during pregnancy include angiotensin-converting enzyme (ACE) inhibitors, iron chelators, and hydroxyurea. The use of hydroxyurea is not recommended and women are advised to avoid conception up to 6 months after their last dose of hydroxyurea due to animal studies showing teratogenicity, and a few case reports of fetal growth abnormality and pre-term birth. For more recently approved SCD-specific therapies, the antioxidant aminoacid L-glutamine is generally considered to be safe, the antisickling agent voxelotor is safe through pregnancy but not recommended during lactation, and there are no data on the anti-adhesive monoclonal antibody crizanlizumab

Care for pregnancy in SCD patients must include specializes antenatal care since complications can occur at early gestational ages. SCD pregnancy requires more frequent follow up including a multidisciplinary team (27, 39, 50): obstetrician, with close fetal surveillance, hematological support, nutritional and psychological assessment, ultrasonographist experienced in maternal-fetal medicine.

It may be convenient that appointments with the obstetrician take place at least monthly in the first and second trimester and more frequently after that, with individual assessment. It is paramount to check immunization status and update it when necessary, taking into consideration that SCD patients require broader coverage for encapsulated bacteria (e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae, Neisseria*). Laboratory checks must include hemoglobin level, hemolytic markers (reticulocyte count and LDH) and infectious screening (especially urinary tract infection and asymptomatic bacteriuria), more frequently than for a low-risk pregnancy.

Anemia during pregnancy is defined by the World Health Organization (WHO) by a hemoglobin level below 11g/dL (5, 6) at any trimester of gestation. Maternal anemia is a common condition even for those women with normal

hemoglobin, especially during the last trimester of pregnancy, when iron needs increase (52). When anemia presents in the first and second trimester, it may contribute to LBW, prematurity and neonatal complications (52, 54, 55). Chronic anemia is a very common feature of SCD and impacts patients from the beginning of pregnancy. However, it is important to assess if there are additional contributors to maternal anemia, such as nutritional deficiencies. Anemia can negatively affect fetal development resulting in a growth restricted fetus (20, 51, 54, 55). The hemoglobin level of these patients can be very low and the need for blood transfusion is common. Therefore, many studies evaluated the benefit of prophylactic blood transfusion during pregnancy.

Red blood cell transfusion aims to improve oxygen carrying capacity. It can be performed through simple transfusion when the goal is to achieve a certain level of hemoglobin, or exchange transfusion (manual or automated erythrocytapheresis) (114) when the aim is to decrease circulating HbS. In acute complications that lead to severe anemia, simple transfusion is usually the choice. Those transfusions aim to increase hemoglobin levels closer to baseline while avoiding hyperviscosity and heart failure (22). Red cell transfusions can also be chronically used for stroke prevention, and acutely to treat acute chest syndrome or multisystem organ failure (22). Exchange transfusion has been used during pregnancy to reduce the occurrence of complications but there is no definitive evidence to indicate it to all patients. If red cell exchange is indicated in pregnancy, reasonable targets would be a HbS less than 40% with hemoglobin level of 10g/dL (75).

A metanalysis in 2015 concluded that prophylactic transfusion was associated with lower maternal mortality, VOCs, pulmonary complications, perinatal and neonatal mortality, and premature delivery but no improvement in preeclampsia and FGR (69). Other studies reinforce the findings of lower maternal mortality (70) and VOCs (70, 71) with prophylactic transfusion. A Brazilian study in 2007 found better fetal outcomes with less fetal growth restriction and less preterm deliveries (115). Nevertheless, there is no consensus over the ideal hemoglobin level during pregnancy or over the best moment to start transfusions in pregnant SCD patients. Some recommend starting transfusions in the second trimester (70) while others start earlier in the first

trimester (73, 74). A more conservative approach is to transfuse pregnant patients who have severe anemia with Hb below 7g/dL, or any level of anemia if signs of impaired fetal growth or fetal distress are observed. To make this decision, providers should also consider that the more transfusions, the greater the risk of alloimmunization, which is about 16-20% in this population (39, 70). Alloimmunization can lead to lifetime difficulty to find compatible blood and delayed hemolytic transfusion reactions (116), the latter sometimes manifesting with hyperhemolisis syndrome, a life-threatening situation in which hemoglobin levels drop to below pre-transfusion levels.

Placental findings in SCD

The placenta is an organ with an adapted surface for oxygen and nutrient exchange between the maternal and fetal circulations (117). Studies in SCD have shown placental abnormalities such as syncytial knots, villous necrosis, congestion, deposits of sickle hemoglobin and intravillous fibrin (57, 118). Increased expression of pro-inflammatory genes in the placenta have been documented suggesting that the organ is exposed to a pro-inflammatory environment and hypoxia (20), and the imbalance of inflammatory substances could favor vaso-occlusive episodes and necrosis (57, 75). Furthermore, abnormal placental size, localization, and adhesion to the uterine wall have also been described in SCD pregnancies. The exact pathophysiology explaining how abnormalities in placental development can contribute to worse perinatal outcomes and complications in SCD is not yet fully understood. However, those abnormalities may increase risk of uteroplacental insufficiency, leading to maternal and fetal adverse outcomes (20).

Fetal surveillance and risk of fetal growth restriction

Fetal growth restriction (FGR) is when the fetus does not reach its biological growth potential and is usually associated to placental insufficiency (64) (55). Those fetuses are at higher risk of adverse outcomes in pregnancy, higher morbidity and mortality, and impaired neurological development (65, 66), which makes recognition and appropriate follow up of such cases essential. FGR is defined by statistical deviations from population-based reference growth curves (67).

For fetal assessment, ultrasound (US) scan is the preferred method to evaluate fetal wellbeing as it can estimate fetal weight (especially important during the third trimester) and detect placental impairment with Dopplervelocimetry and, therefore, it should be part of regular antenatal care. However, due to the heterogeneity among SCD patients, there is no specific recommended protocol for US follow up. We suggest performing US at least once during the first and the second trimester, and then monthly until delivery. That seems like a reasonable approach if there is no major complication and providers should consider shorter scan intervals if necessary. During the third trimester, closer follow up with a two-week interval if there is early suspicion of fetal impairment is acceptable.

Diagnosis and treatment of complications during pregnancy

SCD complications may have distinct presentations and should always be suspected based on patient's history and exam. Symptoms can sometimes mimic common pregnancy discomforts, delaying adequate healthcare.

Vaso-occlusive crises (VOCs) are the most frequent complication and are typically experienced as acute bone or joint pain that starts abruptly. Uncomplicated VOCs last for 4-5 days on average. The severity of pain also varies widely but severe pain often requires admission. The treatment of VOC is based on hydration, analgesia, and treatment of precipitating factors. Common triggers for VOC include dehydration, sudden changes in temperature (including but not limited to cold exposure), infections (including urinary tract infection, pneumonia, acute osteomyelitis, etc.), delayed transfusion reactions, thromboembolic events, acute phase of avascular necrosis, and emotional distress, but many VOCs won't have an identifiable trigger. Controlled fluid management should be enough to reduce blood viscosity but must not be overdone to avoid acute pulmonary edema. There is no scientific evidence to recommend a specific type of intravenous fluid, and providers can freely choose to use normal saline, NaCl 0.45%, Ringer lactate, and others. Analgesia must include non-opioid and/or opioid analgesics depending on the intensity of pain (119). The use of opioids during pregnancy increases the risk of neonatal complications including withdrawal syndrome of the newborn but should not be

considered a contraindication to the use of opioids in this setting. Non-steroidal anti-inflammatory drugs are not recommended after 34 weeks gestation in order to avoid cardiac dysfunction with premature close of arterial canal. Transfusions should not routinely be indicated for VOC and should take into consideration if there is symptomatic anemia, and potential risks associated with the procedure.

Acute chest syndrome is a major complication characterized by a VOC with acute pain in the thoracic region associated with respiratory symptoms and fever, mostly associated with the finding of a new pulmonary opacity, and hypoxemia in severe cases. It is the main cause of adult death with SCD (18). Treatment is symptomatic with analgesia, fluid management, oxygen supplementation for oxygen saturation below 92% and ventilatory support if necessary. Simple transfusion may be indicated in patients with severe anemia (Hb<7g/dL) and red cell exchange transfusion must always be considered for severe cases with hypoxemia. Acute chest syndrome is indistinguishable from pneumonia; therefore, the use of empiric broad spectrum antibiotics is indicated and use of antivirals should be considered, and consultation with an Infectious Diseases specialist is encouraged to discuss options during pregnancy. The association of SCD and pregnancy increases the risk of deep venous thrombosis and pulmonary embolism, so a low threshold to indicate lung CT angiography is encouraged for patients with signs and symptoms of acute chest syndrome.

While preeclampsia is known to be more frequent in SCD patients, the diagnosis of severe preeclampsia with HELLP (“hemolysis, elevated liver enzymes, and low platelets”) syndrome may be challenging. Laboratory abnormalities caused by SCD delay the detection of hemolysis, since lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels are increased by chronic hemolysis, and haptoglobin is already undetectable in SCD. In addition, HbSS patients may have baseline elevated platelet counts due to autosplenectomy, sometimes above 600,000, and HbSC or HbSβ often have splenomegaly leading to mild to moderate chronic thrombocytopenia in the 80-150,000 range. Young patients may already have some degree of microalbuminuria prior to pregnancy, so early evaluation of the urinary sediment is encouraged as a baseline to help detect abnormal proteinuria later in pregnancy. Therefore, patients presenting with signs and symptoms suggestive

of severe preeclampsia, such as headache and abdominal pain, should be carefully evaluated and monitored, and their laboratory evaluation must take into consideration their earlier results. We recommend keeping track of baseline urinalysis, LDH, liver enzymes, and platelet counts and throughout pregnancy to help the diagnosis of preeclampsia and HELLP syndrome in SCD patients. Sickle cell complications such as sickle hepatopathy and hepatic sequestration should be considered as differential diagnosis since they may also lead to increase in liver enzymes and worsening anemia.

Follow-up and childbirth

Vaginal delivery is possible, so SCD should not be regarded as an indication for cesarean section. Labor may occur spontaneously or after induction in SCD pregnant patients. During labor, it is important to be mindful of the patient's hydration status, provide appropriate analgesia since those women are more prone to vaso-occlusive events during stressful situations, and ensure close fetal monitoring.

As a chronic systemic condition, pregnancy in SCD should ideally be planned in order to minimize possible complications. Women of reproductive age and during the postpartum period must be counseled about contraceptive options. According to the WHO, contraceptive methods using only progesterone (pill, injectable, implants or intrauterine device) are category 1, meaning that the method can be used in any circumstance. Combined hormonal contraceptives and copper intrauterine device are category 2, meaning that the method can generally be considered, but should take into account individual risk of thromboembolic events, history of menorrhagia, and patient preference (120).

Psychological and nutritional visits should happen together with obstetric care and puerperium as these patients have higher rates of hospitalization and are more prone to develop depressive episodes (121, 122) and chronic nutritional deficiency (123).

SCD does not constitute a contraindication for breastfeeding, but treatment with hydroxyurea is usually held until the patient is not breastfeeding. In addition, patients who were treated with blood transfusions during pregnancy,

should be evaluated for iron overload by their hematologist, particularly if chronically transfused. Indication for iron chelation therapy will depend on workup with serum ferritin, transferrin saturation, and liver magnetic resonance.

Low bone mineral density is a frequent complication of SCD (124), even in women of childbearing age, and can sometimes remain undiagnosed for many years. Pregnancy and breastfeeding contribute to deplete calcium from the bones (125) and we encourage providers to evaluate patients for osteopenia and osteoporosis with dual-energy X-ray absorptiometry (DEXA) scan after they have finished breastfeeding, and verify vitamin D levels to provide supplementation if needed.

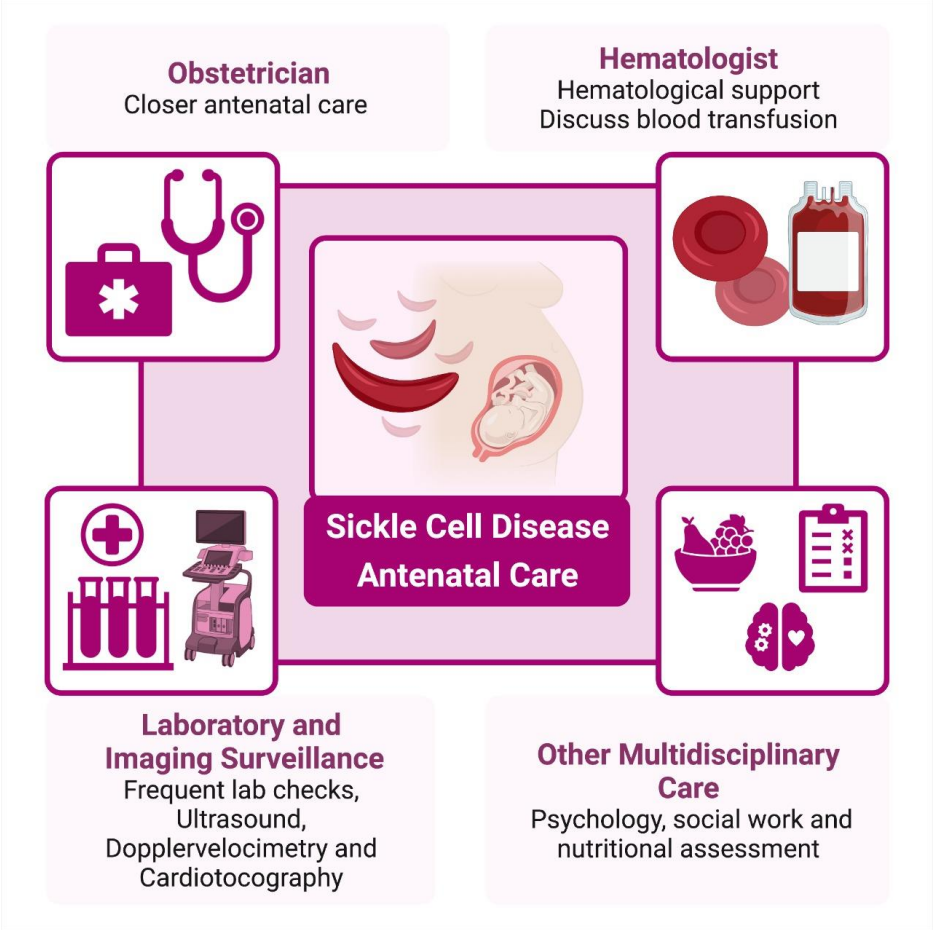

Figure 1: Antenatal care of pregnant women with SCD

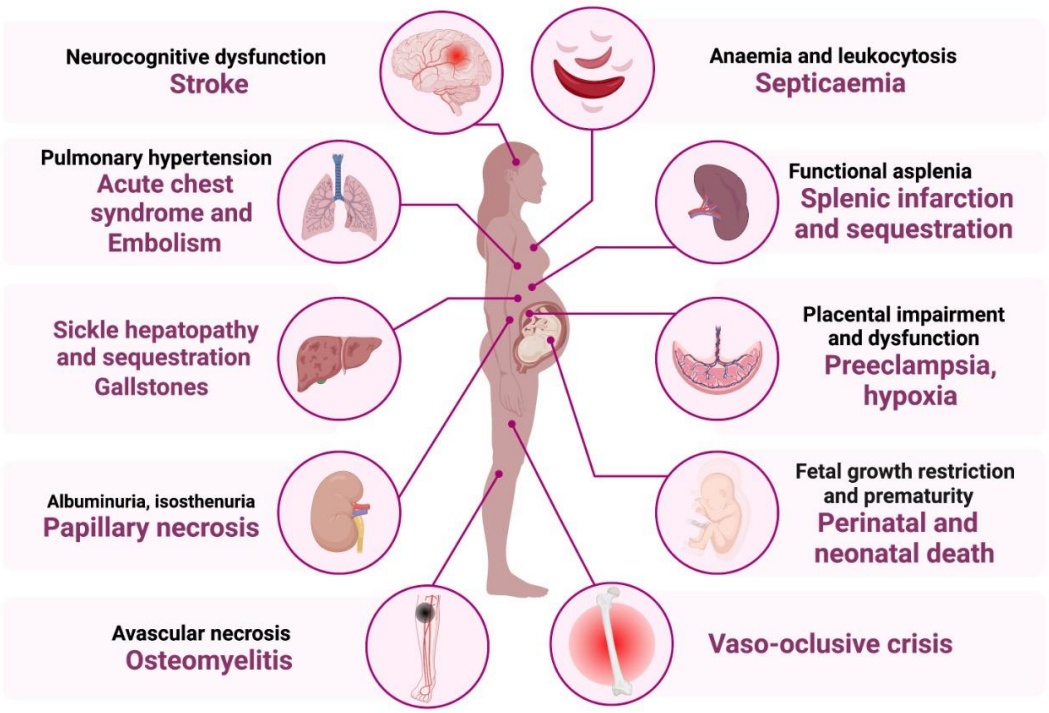

Figure 2: Frequent complications in Sickle Cell Disease (SCD)

Conclusion

SCD is a complex chronic disorder with potential life-threatening complications during pregnancy. Management of pregnant SCD patients requires a multidisciplinary approach to achieve favorable maternal and fetal outcomes, with accurate and timely diagnosis and treatment of its complications.

Acknowledgements:

Figures created with BioRender.com

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4.3 ARTIGO 3

High maternal perinatal morbidity and placental abnormalities in sickle cell disease pregnancies: a single-center retrospective analysis

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Abstract

Background: Sickle cell disease is a heterogeneous group of inherited hemolytic disorders that increases the risk of maternal and fetal complications due to a chronic systemic inflammatory response and increased endothelial damage and vaso-occlusion. The contribution of genotypes to the severity of the outcomes is not completely established. We aimed to compare maternal and perinatal outcomes and placental findings among the three most common genotypes. **Methods:** retrospective study with medical chart review, comparing maternal and perinatal outcomes in HbSS, HbSC disease and sickle-beta thalassemia (HbSβ) among pregnant women followed at a high-risk antenatal care, over a 6-year period. For categorical variables, qui square test (X^2) was performed and for continuous variables, Mann Whitney or Kruskal-Wallis was performed with p-value <0.05 as statistically significant. For cases with available pathology report of the placenta, a descriptive analysis of placental morphological findings was performed. **Results:** We analyzed 62 SCD pregnant women, of which 25 were HbSS (40%), 29 HbSC (47%), and 8 HbSβ (13%). Overall mean maternal age was 27 years, pre-pregnancy body mass index was 22.3, 52% of all women were primiparous and 45% of them started antenatal care at first trimester and these data were not significantly different among the three groups. Hemoglobin level at first medical visit was lower in HbSS (7.7g/dL) than in HbSC and HbSβ (9.7 and 8.4g/dL, respectively, p= 0.01). Maternal complications (worsening anemia, pain crisis, acute chest syndrome, and infection) occurred in 77% of patients, preterm birth in over 30% of cases, cesarean section around 80%, with no statistically significant differences among genotypes. Maternal death occurred in only one HbSS woman. Blood transfusion was needed in 96% of HbSS, 79% HbSC and all HbSβ women (p= 0.08). Ten of 15 evaluated SCD placentas showed abnormal morphological findings, half of them weighed below the 10^{th} centile and 90% presented with maternal vascular malperfusion features. **Conclusion:** SCD remains a condition with high maternal and perinatal morbidity, with increased rates of complications and placental abnormalities regardless of genotype and despite treatment in a specialized reference center. Larger studies are needed to identify risk factors for complications and develop novel strategies to improve outcomes.

Background

Sickle cell disease (SCD) is one of the most prevalent recessive inherited disease worldwide (1) and affects more than 300,000 newborns every year (2-4). In Brazil, more than 2 million people carry the sickle cell gene mutation, and about 25-50,000 people live with homozygote sickle cell anemia (HbSS)(5).

The structurally abnormal hemoglobin S (HbS) is the result of a single nucleotide substitution in the β globin chain. Deoxy-Hb S polymerizes at low oxygen levels, stretching the red blood cell (RBC) membrane and making it less flexible and adherent to endothelial cells (6). SCD shortens the RBC lifespan by having RBCs removed from circulation either by the reticuloendothelial system or through intravascular hemolysis (6), releasing free hemoglobin in circulation. In addition, RBC sickling and increased cell adhesion lead to endothelial and coagulation cascade activation creating a chronic systemic inflammatory response (7).

SCD arises not only from sickle cell anemia (HbSS), but also from double heterozygosity for HbS and hemoglobin C in hemoglobin SC disease, and sickle β thalassemia (HbSβ), caused by combinations between HbS and thalassemic mutations that can be β^0 (absent hemoglobin A synthesis) or β^+(with some residual production of normal hemoglobin A) (1, 2, 6, 8). Some heterozygous presentations of SCD may have less severe outcomes (9, 10), although such women are still at higher risk of complications, especially during pregnancy and postpartum.

Increased pregnancy and postpartum morbidity (2, 9, 11) and mortality (9, 11, 12) in SCD has been recognized and include hypertensive disorders, thromboembolic events, fetal demise, fetal growth restriction, prematurity (13), and maternal death (10, 14). In addition, SCD complications such as pain crisis, acute chest syndrome, increased susceptibility to infections (15, 16), and worsening anemia are frequent in pregnancy (13) and account for recurrent hospitalizations and mortality. Therefore, SCD is a challenging condition that needs a multidisciplinary antenatal approach (17-19).

RBC transfusions are commonly used in SCD in order to treat severe anemia and to decrease the percentage of circulating HbS. During pregnancy, they are often utilized on demand but can also be prescribed prophylactically to prevent SCD complications (11).

Data on pregnancy outcomes across the different genotypes in SCD are still relatively scarce and previously published results varied widely depending on whether studies were conducted in low- or high-income settings. There is also paucity of data on placental abnormalities which are expected in a systemic inflammatory condition such as SCD. Our study aimed to describe maternal and perinatal outcomes and placental findings across different genotypes of SCD in a tertiary center of reference in Southeastern Brazil.

Methods

This was a retrospective study with medical chart review of SCD cases followed at the Women's Hospital, University of Campinas, Brazil, during antenatal care, childbirth and postpartum. This is a reference university hospital in Southeastern Brazil providing specialized care to a surrounding population of 3,100,000 inhabitants and located in the same neighborhood as the SCD adult and pediatric reference centers.

The study protocol was approved by the local research ethics committee (#14092013.4.0000.5404) and individual informed consent was waived since data collection was retrospective, with no further interventions. The STROBE Statement (Strengthening the Reporting of Observational Studies in Epidemiology) checklist was followed in order to standardize our findings considering the recommendations for observational studies (20).

Data was retrieved from medical records of all the women referred to the institution for antenatal care due to SCD (confirmed with hemoglobin electrophoresis) and that had childbirth at the same maternity hospital. Women with childbirth in other institutions were excluded from the final analysis, due to lack of information on maternal and perinatal outcomes. Cases were grouped according to SCD genotype (HbSS, HbSC, or HbSβ) for comparison. The variables analyzed included sociodemographic data (maternal age, skin color, body mass index at first prenatal care visit, previous obstetric history, smoking), maternal outcomes and frequency of complications (worsening anemia, pain crisis, acute chest syndrome, occurrence of preeclampsia), perinatal outcomes (gestational age at delivery, birth weight, 5^{th} minute Apgar score, neonatal complications), placental morphological analysis when available, and need for blood transfusion. Blood transfusion were classified into prophylactic or on demand, according to an institutional protocol established since 2011 that determines the indication for scheduled prophylactic red blood cell transfusion around 28 weeks of gestation for SCD cases. Transfusion was considered prophylactic if there was no indication due to acute complication.

Contingency tables were used for categorical variables and for statistical analysis, and qui square test (X^2) was performed. For continuous variables, the description of mean, standard deviation, maximum and minimum values and for statistical analysis Mann Whitney or Kruskal-Wallis was performed if the comparison was between two or three groups respectively. We considered p-value <0.05 as statistically significant and used the EPI-INFO software for analysis.

Results

Between January 2011 and May 2017, 67 women with SCD were seen at the Women's Hospital due to pregnancy. Of those, 62 gave birth at the same institution and complete data on maternal and perinatal outcomes were available for analysis. According to genotype, 25 (40%) had the homozygous form of the disease (HbSS) and

37 were double heterozygotes with 29 (47%) HbSC and 8 (13%) HbSβ with 7 HbSβ^0 and 1 HbSβ$^+$. Figure 1 shows a flowchart of the cases studied.

Table 1 shows the sociodemographic characteristics of each subgroup, clinical and obstetric background. Non-white skin color was more frequent among HbSS and HbSC group (>50% each group), and 12.5% among HbSβ patients (p=0.11). Mean maternal age was similar in the three groups with an average of 27.1 years. Prevalence of primiparous women was higher in the HbSS genotype (64%) than in HbSC and HbSβ (45% and 38%, respectively), but not statistically significant. Previous cesarean section occurred in 60% of all multiparous women (n=30), 67% in HbSS, 63% in HbSC, and 40% in HbSβ (p= 0.67). Overall, only 45% started antenatal care before 12 weeks of gestation with no differences across genotypes. Hemoglobin level at first medical visit was significantly lower in the homozygous genotype (7.7g/dL) when compared with HbSC and HbSβ (9.7g/dL and 8.4g/dL, respectively, p= 0.01).

Maternal and perinatal outcomes are shown on Table 2. Prematurity was slightly more frequent in the HbSβ group (50%), although not statistically significant (p=0.48). Women underwent induction of labor in 36% of HbSS genotype, 51.72% of HbSC and 62.5% of HbSβ. Cesarean section was the route of delivery in 79.03% of all cases, with 84% in homozygous form of the disease. Near miss was a condition found in more than 20% of cases in all three genotypes. Low birth weight was present in 40% of HbSS group, 31.03% of HbSC group and 37.5% of HbSβ one. Neonatal death occurred only among the homozygous group (2 cases).

SCD-related complications are described on Table 3. Almost all women needed at least 1 RBC transfusion during pregnancy (96% in HbSS, 79.31% in HbSC, and 100% in HbSβ genotype) with a trend towards less transfusion in HbSC (p= 0.08). Prophylactic blood transfusion was implemented for 38% of HbSS group, 65% of HbSC, and 38% of HbSβ (p= 0.15). SCD complications occurred in 84% of HbSS women, 69% of HbSC, and 88% of HbSβ (p= 0.32). Those complications included worsening anemia, pain crisis, acute chest syndrome, and infections. Preeclampsia occurred in 15% of patients and was not statistically different across genotypes. One maternal death occurred in the HbSS group, a non-white woman with late onset of prenatal care, less than 6 medical visits who was hospitalized due to a pulmonary infection at 29 weeks gestation and complicated by septicemia and refractory shock.

Placental pathology was available in 15 cases (8 HbSS and 7 HbSC), with abnormal findings in 10 of them (5 HbSS and 5 HbSC). From the 10 placentas with abnormal findings, 5 placentas were hypoplastic (defined as weight below the 10th centile), 9 showed maternal vascular malperfusion (MVM) (utero-placental hypoxia). The MVM is divided in: global/partial type MVM with accelerated villous maturation in 50%, and segmental/complete type MVM represented by infarctions in 40%. Two of the 9 placentas that showed MVM had both patterns of MVM. Extensive infarcts were

considered the cause of death for two stillbirths, both in HbSC patients. Figure 2 shows representative images of the placental findings.

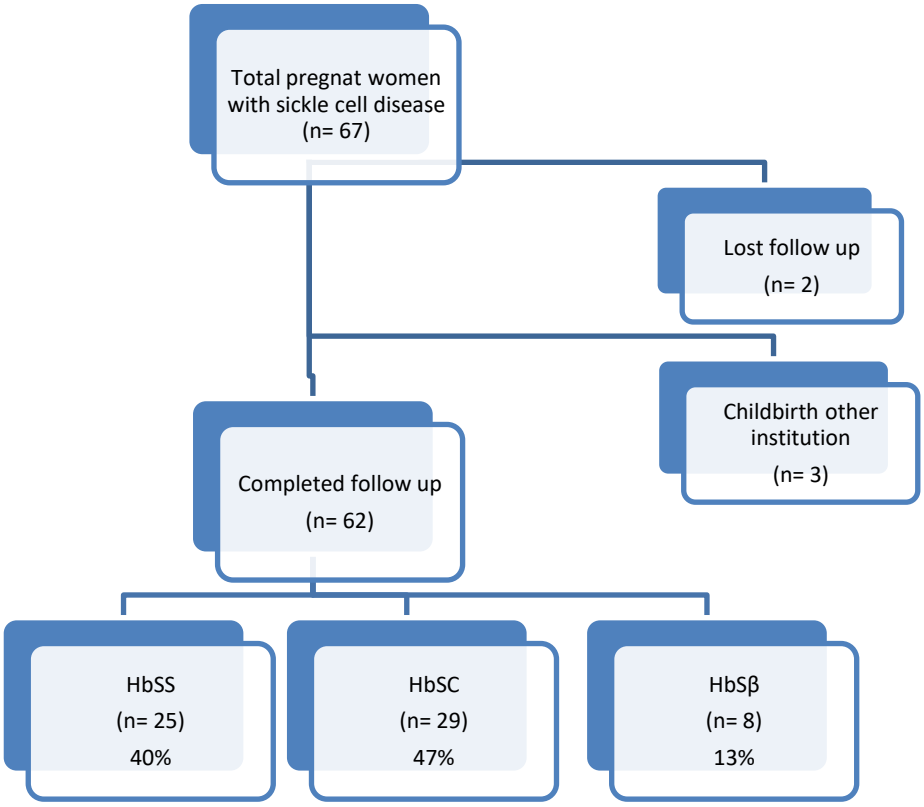

Figure 1. Flowchart of reviewed cases between January 2011 and May 2017

Table 1. Maternal sociodemographic characteristics, clinical and obstetric history

Maternal profile		SCD genotype			
	Total cases	SS (N= 25)	SC (N= 29)	Sβ (N= 8)	*P*
	N (% or SD)	N (%)	N (%)	N (%)	
Mean maternal Age (years)	27.1 (5.3)	25.6 (5)	26.3 (6.4)	29.3 (6)	0.28
Non-white skin color	30 (48)	12 (56)	17 (59)	1 (13)	0.11*
Mean BMI at 1st medical appointment**	22.47 (4)	22.02 (4.2)	23.31 (4.5)	22.09 (3.3)	0.51
Smoking	4 (6)	1 (4)	3 (10)	0 (0)	0.46
Primigravida	32 (52)	16 (64)	13 (45)	3 (38)	0.25*
Previous cesarean section***	18 (60)	5 (71)	10 (63)	3 (30)	0.46
Onset of antenatal care <12 weeks	28 (45)	11 (44)	12 (41)	5 (63)	0.56*
Mean Hb level at 1st medical appointment +	8.6 (1.1)	7.7 (1.1)	9.7 (1.0)	8.4 (1.3)	<0.01

*Qui Squared test; **: on the day of first visit; ***: only women with previous pregnancy (n=30, 9 HbSS, 16 HbSC, 5 HbSβ); + missing data for 3 HbSS, 6 HbSC, 1 HbSβ

Table 2. Maternal and Perinatal Outcomes of Sickle Cell Disease patients according to genotype

Maternal and Perinatal Outcomes	Total cases N (%)	SCD genotype			
		SS (N= 25) N (%)	SC (N= 29) N (%)	Sβ (N= 8) N (%)	*P*
Preterm birth	20 (32)	8 (32)	8 (28)	4 (50)	0.48
Induction of labor	29 (47)	9 (36)	15 (52)	5 (63)	0.32
Route of delivery					0.73
- Cesarean	49 (79)	21 (84)	22(76)	6 (75)	
- Vaginal	13 (21)	4 (16)	7 (24)	2 (25)	
Maternal complication postpartum*	14 (23)	6 (24)	6 (21)	2 (25)	0.94
Near miss criteria**	14 (22.58)	5 (20)	6 (21)	3 (38)	0.55
Maternal mortality	1 (2)	1 (4)	0 (0)	0 (0)	n/a
5-minute Apgar <7	5 (8)	1 (4)	4 (14)	0(0)	0.28
Mean birth weight in grams (SD)	2,562 (715)	2,479 (628)	2,575 (815)	2,632 (701)	0.48
Low birth weight	22 (35)	10 (40)	9 (31)	3 (38)	0.78
Neonatal complication***	13 (21)	6 (25)	6 (22)	1 (13)	0.70
Perinatal death	5 (8)	3	2	0	n/a
Placental morphologic analysis	15 (24)	8	7	0	n/a
-abnormal findings[ɬ]	10 (16)	5	5	0	

GA: gestational age; g: grams; *hemorrhage, infection, sickling and others; ** admission at intensive care unit, red blood cell transfusion due to acute hemorrhage, eclampsia, renal, or cardiac complications; *** admission at neonatal intensive care unit, mechanical ventilation, neonatal surgery, need for vasopressors (1 missing data in SS and 2 in SC group); n/a: not applicable; ɬ: accelerated villous maturation, infarctions, placental hypoplasia.

Table 3. Sickle cell disease complications among pregnant women during antenatal care, childbirth, and postpartum.

SCD complications	Total cases N (%)	SCD genotype			
		SS (N= 25) N (%)	SC (N= 29) N (%)	Sβ (N= 8) N (%)	*p*
Any red blood cell transfusion	55 (89)	24 (96)	23 (79)	8 (100)	0.08
Prophylactic transfusion	27 (44)	9 (38)	15 (65)	3 (38)	0.15
Overall complications	48 (77)	21 (84)	20 (69)	7 (88)	0.32
- WA		7	4	1	
- Pain crisis		13	12	5	
- ACS		6	4	3	
- infection		14	10	3	
Preeclampsia	9 (15)	3 (12)	4 (14)	2 (25)	0.65
Mean days of hospital admission (SD)	7.5 (9.7)	7.2 (6.5)	4.7 (6.8)	10.7 (15.9)	0.18

WA: worsening anemia; ACS: acute chest syndrome

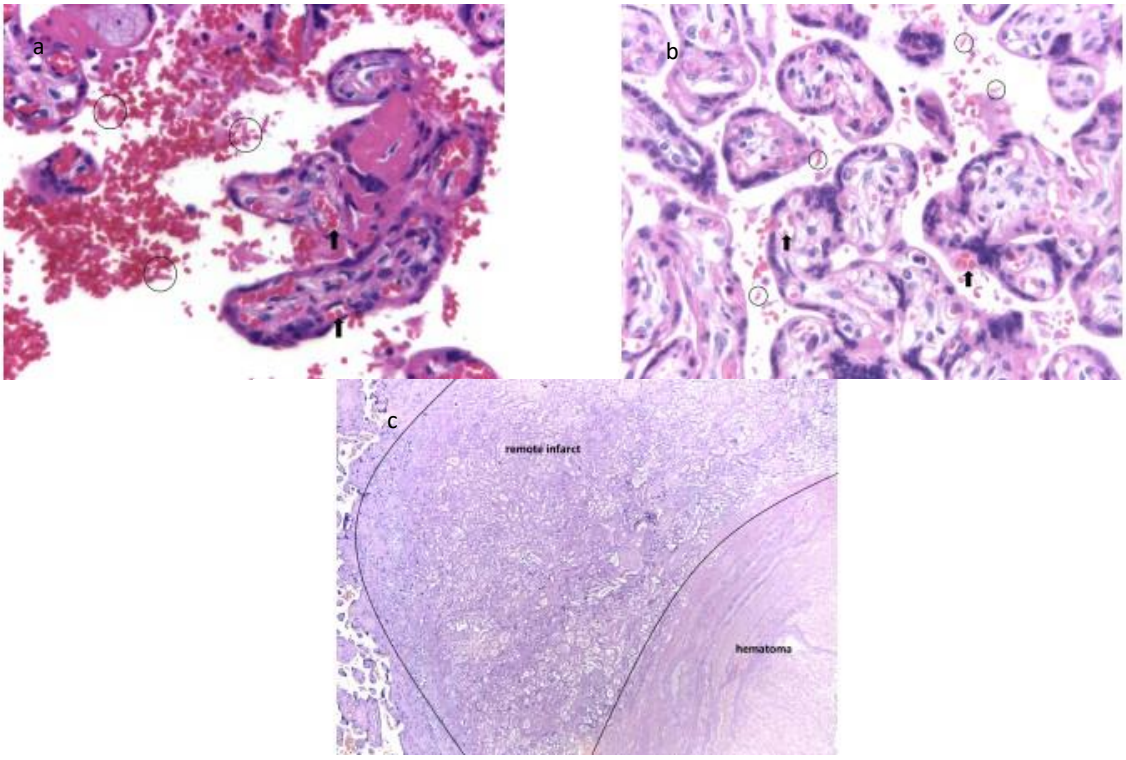

Figure 2: Representative abnormal placental findings in sickle cell disease (SCD) among pregnant women.

Panels **a** and **b**: HbSS with hypoplastic, term placenta and many sickled red blood cells in the intervillous space (circles). Normal fetal red blood cells in the villous vessels (arrows). HE, 400x; Panel **c**: HbSC with hypoplastic, term placenta with a remote centrally hemorrhagic villous infarct (infarction hematoma), HE, 40x.

Discussion

Sickle cell disease has variable impact on the outcomes of pregnancy. This study aimed to evaluate maternal and perinatal outcomes of different SCD genotypes.

HbSS in usually the most frequent genotype in the SCD general population, followed by HbSC and then HbSβ , but it can vary according to country and region (21). In this study of SCD in pregnancy, HbSC genotype was the most frequent (47%), differently from previous reports in which HbSS was predominant (11, 22, 23), which has allowed us to better characterize outcomes in this subtype of SCD. HbSβ genotype accounted for 13% of our patients and was mostly composed by the HbSβ^0 subtype (7/8 patients). HbSβ^0 is the most severe form of HbSβ because it also causes the production of over 90% of HbS, similarly to what is found in HbSS, and both conditions are clinically indistinguishable. Therefore, it was unsurprising that HbSβ translated into poor outcomes not very different from the HbSS group in this study. Non-white skin color was found in the majority of HbSS and HbSC cases, in agreement with previous studies, as SCD is more common in African-derived populations (24). The relatively higher prevalence of Caucasian ethnicity in our HbSβ group can be explained by frequent population admixture in the Brazilian population with the co-inheritance of β thalassemia mutations of predominantly Portuguese and Italian origin.

It is important to notice that less than 50% of SCD women started antenatal care during the first trimester. We believe that unplanned gestation and difficulties in transition from pediatric to adult care may play a role in contributing to this. SCD being a chronic disease with high morbidity associated with pregnancy, a planned gestation, with early scheduled antenatal care would be key towards more adequate healthcare (25, 26).

We found a relatively higher percentage of primiparous women among the HbSS genotype (64%) when compared to HbSC and HbSβ (44 and 37%, respectively). While this was not statistically significant due to the low numbers, we hypothesize that a higher morbidity of HbSS disease prior to pregnancy may contribute to postponing the decision of some women to undergo gestation (reproductive planning). A Brazilian study about reproductive issues in SCD did not find differences in age of first pregnancy, number of pregnancies, or number of living children among the three genotypes (27), supporting the hypothesis of personal decisions in delaying pregnancy.

Prematurity is a common adverse outcome in SCD patients, and it is mostly medically indicated. Worldwide prematurity rate is 11.1% according to the World Health Organization (WHO) (28) and Brazilian national data estimate an incidence from 9.9 to 12.3% (29). SCD rates of prematurity vary from 4.1% in a study with 37 women in Turkey, (30) to 50.4% in a study with 109 women in the United Kingdom (9, 10). Our study presented high rates, with an overall incidence of 32%, either due to maternal condition or fetal distress, in agreement with previous publications. Half of preterm deliveries happened before 34 weeks of gestation, accounting for more severe adverse outcomes among neonates. More than one third of the babies in our study had low birth weight and 8% received Apgar score below 7 at the 5^{th} minute. We found no significant differences in prematurity among genotypes while a study from UK found prematurity rate of 47.1% in HbSS and 20.5% in HbSC groups (p=0.01) (9). This discrepancy may be due to the smaller number of patients in our study, which analyzed patients from a single tertiary hospital but suggests that providers should not rely on genotype to predict chances of prematurity.

Labor induction was attempted in 46% of our patients with more than a third in the HbSS women and more than 50% in the HbSC or HbSβ. That was higher than another study that compared HbSS to HbSC women, with an overall induction of labor rate of 39.44%. In both studies the difference between the genotypes was not significant (10). During induction and labor, it is important to monitor hydration, symptoms of possible complications and methods for pain relief, including early analgesia. Evaluating the need of blood transfusion prior to induction is also recommended, as some patients may have peculiarities in finding compatible blood.

We observed a high rate of cesarean section as the route of delivery, 79% of all cases and 84% in HbSS. A review of literature describes rates from 16 to 91% (31) and another study from UK found an overall prevalence of 37.61% with 52.9% in homozygous genotype (10). We believe this finding reflects not only the severity of cases, but may stem from a local understanding in the Brazilian population, known as a

country with one of the highest cesarean rates in the world (32). Therefore, this should not be interpreted as evidence that SCD leads to the need for cesarean section more often than other comorbidities during pregnancy, nor does it mean that SCD is an indication for cesarean section in our population.

Comparing our findings to another study in the same institution with a case series of seven patients from 2002 to 2004, we notice the increased number of pregnancies (7 pregnancies in 3 years versus 67 pregnancies in 6 years) and the increased presence of the HbSC genotype. We also found the persistence of the high prevalence of complications and cesarean section showing that, despite almost 2 decades of difference, new studies and treatments, the frequency of complications remains high. The number of cases increased by almost ten times, possibly due to easier access to a referral center or a real increase in patients surviving to childbearing age due to more effective treatment and follow-up of SCD during childhood and adolescence, preserving fertility (33).

Nevertheless, SCD is a condition that affects women in all socioeconomic conditions and in high- and low-income countries. The disease is an important risk factor for hypertensive disorders, fetal growth restriction, infections, thromboembolic events and maternal death (34-36). Our overall preeclampsia rate was 15%, with no difference within the genotypes. This value is higher than reported for a population without hemoglobin disorders, but similar to that described in a study in the USA (37) and lower than another Brazilian study that found higher rates of preeclampsia among all three genotypes (22). Our lower frequency of preeclampsia may be due to a local protocol of prophylactic use of calcium and acetylsalicylic acid for all SCD women as they are known to be at higher risk for hypertensive complications, along with the frequent use of prophylactic RBC transfusion programs (scheduled transfusion around 28 weeks of gestation). Further studies are needed to define the real individual impact of each intervention. A metanalysis in 2015 found more than 2-fold risk for preeclampsia in SCD patients and a 5-fold risk for eclampsia in the HbSS group (9). A retrospective cohort with 344 women with SCD demonstrated a 4-fold risk for severe preeclampsia for those with SCD (36).

Our data are in accordance with studies in other parts of the world describing overall maternal morbidity mainly due to pain crises, acute chest syndrome, infections, and severe anemia with need for blood transfusion (2, 9).

Maternal complications occurred in 77% of patients, with no difference according to genotype and almost 90% received RBC transfusions, of which 44% were prophylactic. There was a trend towards a difference among groups in the need for transfusion, suggesting that HbSC patients required less transfusions, which is expected since anemia is also less severe in HbSC than in other genotypes. Chronic anemia is a characteristic of the disease and blood transfusions are frequent necessary for these patients as a severe, non-treated anemia can have negative consequences since the beginning of pregnancy. There is lack of evidence to recommend specific hemoglobin thresholds during pregnancy, so monitoring for fetal complications, frequency of

maternal complications, and prior history of complicated pregnancy should be considered in the provider's decision to transfuse on demand or prophylactically.

Pain crisis had a prevalence of 48% with higher frequency in HbSβ group (62%), differently from another Brazilian study that found higher rates of pain crisis in HbSS (22). That may be secondary to our study being enriched in more severe HbSβ^0 cases. In 2016, a metanalysis described the frequency of pain crisis ranges from 0.4% to 77.8% and pulmonary complications (including acute chest syndrome) ranges from 0.4 to 29.6% (31). In our study, acute chest syndrome complicated 19% of pregnancies and according to literature is one of the main causes of maternal death (38).

Maternal near-miss rate was 23%, meaning that 1 woman in five almost died in pregnancy and/or postpartum, underscoring the severity of the disease and the importance of a referral for high-risk health facility to support these women. We found one maternal death in the HbSS group (overall rate of 1.6% among all SCD women), in contrast with the past decades when maternal mortality rate was around 11% (39). This can be attributed to an improvement in maternal care and vigilance with a better understanding of clinical presentation of SCD-related complications and life-threatening situations.

While we had a limited number of placental specimens be analyzed for morphologic abnormalities, two-thirds of the cases showcased the effects of SCD vaso-occlusion in the placenta causing hypoplasia and infarcts. Such abnormalities were found regardless of genotype, suggesting once again that genotype is a poor predictor of complications during pregnancy. Since our patients were already broadly treated with transfusions and acetylsalicic acid, and currently available treatments for SCD are contraindicated during pregnancy, other interventions need to be developed. Further larger studies are warranted to investigate risk factors for placental abnormalities to propose effective treatment and prevention.

This study has limitations: it was retrospective, based on medical records that are not always standardized. The number of cases is limited as data were collected in only one tertiary medical service and a few patients did not deliver at the same institution. On the other hand, an important strength of this study is the setting: being the only tertiary center providing specialized care for SCD patients, we were able to capture over 90% of all SCD pregnancies in the metropolitan area during a period of seven years. We were also able to compare outcomes separated into the 3 main SCD genotypes and reviewed placental findings which have not been previously reported in other studies. Finally, the same institutional protocols to treat SCD during pregnancy were applied to all patients, reducing the effects of heterogeneous care when comparing different genotypes over time.

In summary, we demonstrated that SCD remains a disease with high maternal and perinatal morbidity, with high rates of complications in all genotypes and with frequent structural placental abnormalities. Larger prospective studies are needed to identify risk factors for complications and develop new strategies to improve outcomes.

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5. DISCUSSÃO

Esta dissertação de Doutorado apresenta o resultado de uma pesquisa de uma condição genética pouco frequente, de prevalência variável dependendo da região do país e do mundo e com múltiplas formas de apresentação clínica e evolução, o que dificultou a pesquisa de estudos que tivessem metodologia semelhante e resultados comparáveis.

Primeiramente, antes de avaliar diretamente a doença falciforme, optamos por fazer um estudo sobre a anemia na gestação e seu impacto na morbidade materna e fetal/ neonatal, já que a anemia é um fator tão prevalente mundialmente, com incidência estimada em 41.8% nas gestantes de acordo com a Organização Mundial de Saúde (OMS), variando entre países de alta e baixa renda (5, 6, 80, 88). Dados nacionais acerca de prevalência de anemia são escassos. A anemia é também um dos fatores de risco associado a prematuridade, ruptura prematura de membranas ovulares, baixo peso ao nascer, cesárea, morbidade neonatal, hipertensão gestacional, pré-eclâmpsia, entre outros (5-8, 89, 90) e dependendo da sua origem pode ser prevenida e tratada ainda na gestação, minimizando seus riscos. Comparamos esse impacto que a anemia pode acarretar na morbimortalidade entre as gestações a termo e pré-termo e entre esse último, nas diferentes causas de prematuridade (espontânea, ruptura de membranas e por indicação médica). Esse estudo foi feito a partir de uma análise secundária do banco de dados do Estudo Multicêntrico de Investigação em Prematuridade (EMIP). Para análise da anemia nos grupos, tivemos que eliminar cerca de 10% das pacientes de cada braço do estudo (termo e pré-termo), devido à falta de informações acerca do nível de hemoglobina (não constava nos registros médicos pré-natais ou a paciente não

fez os exames). A prevalência geral de anemia foi de 31,7%, valor este significativamente menor que o estimado mundialmente pela OMS. Essa diferença pode ser devido às condições das regiões onde os estudos foram conduzidos, subnotificação de casos, baixa adesão ao pré-natal ou então a uma combinação desses fatores. Comparativamente, a anemia foi maior no grupo pré-termo (33,23%) que no grupo termo (27,74%) e dentre o grupo dos pré-termos, a ruptura prematura de membranas foi o que apresentou maior frequência de anemia com 36,3%. A maior prevalência no grupo pré-termo pode também estar associada a um menor tempo de suplementação de ferro e, portanto, menor tempo para correção dos níveis de hemoglobina. Nosso estudo encontrou associação positiva ainda entre anemia e prematuridade em idade materna abaixo de 20 anos, mulheres não brancas, moradoras da região nordeste do país, menos de 8 anos de escolaridade, presença de outro filho com menos de 5 anos em casa, menos de 6 consultas pré-natal. Esses fatores estão em concordância com a literatura (6, 80, 95, 96) e sugerem baixo nível socioeconômico.

A prematuridade é uma condição multifatorial, que custa milhões de dólares ao sistema de saúde mundial e, apesar dos esforços, estudos e medidas que visam reduzir sua incidência, não houve diminuição efetiva em suas taxas. A alta prevalência de anemia, principalmente nos países de baixa renda, e sua associação com prematuridade, pode ser um ponto para novos estudos visando aperfeiçoar o cuidado pré gestacional em mulheres em idade reprodutiva e pré-natal, visando minimizar o impacto negativo da qualidade nutricional das pacientes nesses locais para também reduzir o custo, impacto negativo e morbidade neonatal advindas da prematuridade.

Devemos lembrar que existem causas não modificáveis para anemia e, nesses casos, cuidado redobrado no pré-natal é mandatório. É o caso da doença falciforme, condição genética de prevalência variável entre os diversos continentes (por exemplo 0.49 a cada 1000 nascidos vivos nas Américas, 0.07 na Europa e 10.68 na África) (15) e mesmo dentro do território nacional, variando entre 1 a cada 650 nascidos vivos na Bahia e 1 a cada 13500 no Rio Grande do Sul (109). Os estudos normalmente são retrospectivos e com pequeno número de sujeitos devido à baixa frequência da doença, dificultando a padronização dos estudos disponíveis e até do acompanhamento dessas pacientes entre os diferentes centros de referência. Além disso, as populações de cada local de estudo podem ser extremamente diferentes (fator socioeconômico, hábitos de vida, escolaridade, acesso a centros de referência e banco de sangue para seguimento e tratamento das complicações da doença), tornando a doença falciforme um desafio para o paciente e para a equipe que o acompanha. Essa divergência nos estudos, bem como a dificuldade de realização de estudos prospectivos com número mais significativo de pacientes nos motivou a realizar o estudo de revisão sobre o tema. Optamos por tabular os estudos das metanálises apenas, pois representam número maior de sujeitos e avaliação estatística mais sólida.

A doença falciforme advém de uma mutação em um nucleotídeo no códon 7 do gene da beta globina que culmina na troca do aminoácido ácido glutâmico por valina. Se ambos os genes carregam essa mutação, a doença se apresenta em homozigose (HbSS) e é conhecida popularmente como anemia falciforme. Atualmente no Brasil cerca de 25 a 50 mil pessoas convivem com essa forma da doença que é considerada a mais severa. As apresentações em heterozigose

ocorrem quando essa mutação se associa a outra mutação da hemoglobina. As mais frequentes e estudadas são a hemoglobina C, formando o padrão HbSC e a β, formando a HbSβ. No geral essas são apresentações mais brandas da doença, exceto no ciclo gravídico-puerperal, em que podem ocorrer complicações ameaçadoras da vida.

Os portadores de doença falciforme normalmente apresentam complicações da doença quando expostos a baixos níveis de oxigenação, pois a hemoglobina S se polimeriza nessas condições, ou seja, assume um formato diferente do habitual, mais rígido e alongado. Associa-se ainda à polimerização, ativação endotelial, de fatores plasmáticos (fatores de coagulação, fibrinogênio e moléculas inflamatórias) (22) e mecanismos endógenos para remoção da célula alterada que perdeu a função de transporte de oxigênio, podendo culminar em complicações isquêmicas e inflamatórias. Esses mecanismos fazem parte da fisiopatologia da doença de anemia hemolítica e crises vaso-oclusivas (17, 20-22). Assim sendo a doença pode acometer praticamente todos os órgãos e sistemas, em gravidade variável e em qualquer fase da gestação.

Os avanços nos estudos genéticos e conhecimento mais detalhado da fisiopatologia da doença proporcionaram diagnóstico mais precoce e melhores opções de tratamento para os pacientes, melhorando a qualidade de vida e aumentando a sobrevida. Assim, um número maior de mulheres chega à idade reprodutiva em condições de engravidar, seja por desejo ou por falta de planejamento familiar.

A gestação, por ser um estado pró-coagulante, pode agravar as complicações já próprias da doença falciforme, e os estudos reconhecem que é mandatório um acompanhamento multidisciplinar rigoroso em centros de

referência. Complicações frequentes na gestação incluem crises álgicas, infecções, piora da anemia, complicações pulmonares, eventos tromboembólicos, falência renal e até morte materna (80, 105, 107). O feto também sofre consequências desse estado relativo de hipóxia e pode apresentar restrição de crescimento fetal, baixo peso ao nascimento, prematuridade e aumento da mortalidade perinatal (10, 79, 81, 107). Além disso, há risco maior de deficiências nutricionais e episódios depressivos devidos não só à própria doença, mas também às hospitalizações frequentes, necessitando, portanto, de acompanhamento psicológico e nutricional.

A maioria dos estudos concorda com o aumento da morbidade e mortalidade materna como: aumento de incidência de pré-eclâmpsia, complicações pulmonares, internações durante a gestação, parto cesariana, transfusões sanguíneas, e aumento da morbidade e mortalidade fetal com maior risco de restrição de crescimento fetal, parto prematuro, alteração neurológica. Entretanto, alguns pontos ainda necessitam de estudos adicionais como a transfusão profilática, que visa à manutenção de menores taxas da hemoglobina S e relativa estabilidade dos níveis de hemoglobina. Alguns estudos mostram redução das complicações, porém outros não encontraram o mesmo desfecho, além de aumentar o risco de aloimunização pelas repetidas transfusões.

Outra dificuldade no estudo da doença falciforme é a heterogeneidade nas formas de apresentação da doença e suas complicações, sendo desafiador a padronização dos achados laboratoriais patológicos, quadro clínico e desenho dos estudos. As análises dos desfechos e complicações nos diferentes genótipos nem sempre estão disponíveis, tornando a avaliação dos estudos ainda mais árdua, já que parte deles avalia a doença falciforme como um todo e

outros analisam os genótipos separadamente. A forma homozigótica da doença (HbSS) é comumente a mais severa, porém na gestação as demais formas podem assumir grau de severidade semelhante, e isso nos motivou a realizar o terceiro estudo que comparou os desfechos maternos, perinatais e avaliação placentária nos diferentes genótipos da doença falciforme: HbSS, HbSC e HbSβ.

A placenta é uma estrutura imprescindível na gestação e com informações moleculares ainda pouco estudadas. O ambiente pode influenciar alterações morfológicas placentárias e seus fenótipos moleculares e seu estudo mais aprofundado poderia ajudar a elucidar maiores detalhes da fisiopatologia de doenças, incluindo a doença falciforme. As placentas coletadas e armazenadas no biobanco seriam utilizadas para avaliação molecular e comparação desses achados com o desfecho dos casos e uma possível associação com o genótipo da doença. Porém essa parte do estudo não pode ser concluída devido à pandemia causada pelo novo coronavírus devido à dificuldade de trabalho de pesquisa presencial e em grupo e também da disponibilidade de matéria prima. Porém essas placentas estão armazenadas de forma a poderem ser retomadas suas avaliações em momento oportuno e, talvez identificar novas informações que possam aprimorar o cuidado com essas pacientes. Foi desenvolvido estudo inicial pelo grupo (Anexo 5).

6. CONCLUSÃO

6.1 A prevalência de anemia geral no estudo foi de 31.7%, sendo 27.74% do grupo com parto de termo e 33.23% no grupo pré-termo ($p<0.01$). Nesse último, 36.3% foram decorrentes de ruptura prematura de membranas, 32.79% por trabalho de parto prematuro espontâneo e 31.27% por indicação médica da resolução da gestação. Nos casos pré-termos, anemia se associou com baixa escolaridade, presença de filho com idade inferior a 5 anos, início tardio do pré-natal, menos de 6 consultas de seguimento, complicações neonatais (sepse, necessidade de suporte ventilatório), neonatos pequenos para idade gestacional, Apgar no 5º minuto abaixo de 7 e morte neonatal.

6.2 A doença falciforme se associou a complicações gestacionais como anemia hemolítica, crises dolorosas vaso-oclusivas, infecções, complicações pulmonares, parto via cesariana, pré-eclâmpsia e morte materna. Restrição de crescimento fetal, baixo peso ao nascer e aumento da mortalidade perinatal foram as condições fetais associadas.

6.3 A doença falciforme é uma condição de alta morbidade e mortalidade, com 32% de parto pré-termo, 23% de *near miss* materno, 79% de parto cesárea. O genótipo homozigoto (HbSS) apresentou maior número de mulheres em sua primeira gestação, maior necessidade de transfusão sanguínea, maior frequência de parto via cesariana e menores níveis de hemoglobina ao início do pré-natal, entretanto não houve diferença estatística significativa na maioria das variáveis comparadas. As placentas apresentaram alterações estruturais frequentes e não relacionadas com o genótipo da doença falciforme.

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8. ANEXOS

8.1 Anexo 1- Termo de Consentimento Livre e Esclarecido (Estudo Multicêntrico de Investigação de Prematuridade - EMIP)

Termo de Consentimento Livre e Esclarecido - CONTROLE

Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal

Estudo Multicêntrico de Investigação em Prematuridade - EMIP

Eu, _____, ____anos, portadora do documento de identidade RG _____, declaro estar ciente dos objetivos do estudo "Estudo de prevalência de prematuridade e avaliação de fatores de risco e formas de conduta em gestações com parto pré-termo", que está sendo realizado no Centro de Atenção Integral à Saúde da Mulher (CAISM/UNICAMP). Fui informada que o estudo procurará avaliar condições que estão associadas com o parto antes do tempo (parto pré-termo). As informações obtidas da minha gravidez e do meu parto serão comparadas com as informações de mulheres que tiveram partos antes do tempo (9 meses). Para isso, fui informada que serei submetida a um questionário com várias perguntas feitas por um pesquisador que se apresentou para mim e me explicou o que será feito para saber as informações necessárias e que assina este documento. Este questionário vai ser preenchido assim que der minha permissão, durante minha internação depois do parto, sendo, também, necessário pegar as informações que estão no meu prontuário e no de meu (minha) filho (a). O tempo para responder às perguntas do questionário deverá ser de aproximadamente 20 minutos. Estou ciente de que este tipo de estudo não poderá trazer benefícios imediatos para mim ou para o (a) meu (minha) filho (a), mas que as informações obtidas poderão ajudar futuramente outras mães no sentido de tentar conhecer melhor as causas do parto antes do tempo e das formas de seu tratamento. Fui informada que meu nome e de meu filho não serão divulgados, mantendo-se o sigilo das informações, não havendo qualquer associação de meu nome e de meu filho com os resultados obtidos. Também fui informada que minhas informações serão analisadas em conjunto com as de muitas outras mulheres do país, para saber os motivos do parto antes do tempo. Estou ciente de que poderei me recusar a participar do estudo a qualquer tempo, sem que isso signifique qualquer problema para meu atendimento no Hospital e de meu filho. Qualquer dúvida que surgir, posso esclarecer com o pesquisador ou com o Comitê de Ética em Pesquisa do Hospital (CEP).

Desta maneira, considero-me esclarecida do que será estudado e declaro consentir com a coleta das informações.

Nome da paciente:

Nome do responsável legal (menor de idade):

Nome do pesquisador responsável: Renato Passini Júnior – Tel. (19) 3521 9304

Telefone do CEP do CAISM/UNICAMP: (19) 3521 8936

Telefone do CEP Local: _____

8.2 Anexo 2- Parecer do Comitê de Ética em Pesquisa (Estudo Multicêntrico de Investigação de Prematuridade - EMIP)

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 08/09/09.
(Grupo III)

PARECER CEP: N° 704/2009 (Este n° deve ser citado nas correspondências referente a este projeto)
CAAE: 0564.1.146.000-09

I - IDENTIFICAÇÃO:

PROJETO: **"ESTUDO MULTICÊNTRICO SOBRE A PREMATURIDADE NO BRASIL".**
PESQUISADOR RESPONSÁVEL: Renato Passini Júnior.
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 07/08/2009
APRESENTAR RELATÓRIO EM: 08/09/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar a prevalência de partos pré-termo numa Rede de Instituições Hospitalares do Brasil, aferindo suas principais condições causais, fatores de risco associados, normas de atendimento e morbimortalidade perinatal.

III - SUMÁRIO

Pesquisa composta por um estudo de prevalência, de corte transversal multicêntrico e um estudo de caso-controle aninhado, a serem implementados em 27 unidades obstétricas de referência nas diversas regiões geográficas do Brasil (Região Norte – 1; Nordeste – 10; Centro-Oeste – 1; Sudeste – 13; Sul – 2). Para o estudo de prevalência os pesquisadores principais e os pesquisadores locais deverão realizar vigilância prospectiva, durante um período de seis meses, de todas as mulheres internadas nessas unidades para parto, para a identificação dos casos de parto pré-termo e suas principais causas. Nos primeiros três meses do estudo, além da avaliação da prevalência do parto prematuro e de suas causas, será feita uma análise de eventuais fatores associados ao parto prematuro, comparando mulheres que tiveram o parto pré-termo com aquelas que tiveram recém-nascidos de termo. Para o estudo de prevalência serão avaliados 37.000 partos (termo e pré-termo), correspondendo a aproximadamente metade dos partos ocorridos no total das instituições participantes em doze meses. Para o estudo de caso-controle foi estimado um tamanho amostral de 1.055 mulheres em cada grupo (casos e controles). O total de partos pré-termo avaliados, incluindo o estudo de prevalência e o caso-controle, corresponderá a 3.600. Os dados serão coletados através de questionário aplicado após o parto, codificados em formulário eletrônico e enviados a um banco de dados central. Análise de dados: A análise dos dados será feita por sub-grupos de acordo com a época da ocorrência do parto pré-termo, suas causas prováveis, as opções de terapêuticas adotadas e resultados neonatais obtidos, estimando-se as respectivas taxas, razões e riscos relativos para os possíveis preditores. Com os resultados encontrados, pretende-se conhecer melhor o nascimento pré-termo no Brasil, seus principais fatores de risco sociais e biológicos, bem como fundamentar ações de política de saúde e dar início a ensaios clínicos abordando as estratégias de prevenção e tratamento das condições causais de partos pré-termo, que tantos agravos físicos e emocionais traz para essas crianças e suas famílias.

IV - COMENTÁRIOS DOS RELATORES

Após respostas às pendências, o projeto encontra-se adequadamente redigido e de acordo com a Resolução CNS/MS 196/96 e suas complementares, bem como o Termo de Consentimento Livre e Esclarecido.

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII- DATA DA REUNIÃO

Homologado na VIII Reunião Ordinária do CEP/FCM, em 25 de agosto de 2009.

Steiner

Prof. Dr. Carlos Eduardo Steiner
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM/UNICAMP

8.3 Anexo 3 – Parecer Comitê de Ética em Pesquisa (estudo doença falciforme)

UNICAMP - CAMPUS CAMPINAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação placentária e ultrassonográfica de gestantes com doença falciforme
Pesquisador: Maria Laura Costa do Nascimento
Área Temática:
Versão: 1
CAAE: 74967717.4.0000.5404
Instituição Proponente: Hospital da Mulher Prof. Dr. José Aristodemo Pinotti - CAISM
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.308.748

Apresentação do Projeto:

Estudo de coorte retrospectivo com avaliação dos laudos de exames US e resultados anatomopatológicos das placentas coletadas de pacientes acompanhadas no pré natal especializado com doença falciforme de 2011 a maio 2017. Esses dados serão
correlacionados com dados maternos (agravamento da anemia, infecções, crise álgica, síndrome torácica aguda, morbidade materna grave ou near
miss, óbito materno e pré eclampsia) e fetais (peso de nascimento, idade gestacional do parto, Apgar e ocorrência de óbito neonatal) Estima-se em torno de 40 casos no período. Para avaliação ecográfica, será utilizado como referência de normalidade de peso fetal os dados do Fetal Growth Study (FGS), para comparação com parâmetros de normalidade, por idade gestacional.Análise dos Dados: Todos os dados coletados serão inseridos em um Banco de Dados constituído para o estudo, no programa Excell for Windows e analisados com auxílio do Epi Info 7. Inicialmente será feita uma análise descritiva, com prevalência dos diversos casos de doença falciforme (diferentes genótipos), resultados maternos, perinatais e também prevalência de alterações de Dopplervelocimetria e morfologia placentária. As variáveis contínuas serão apresentadas em média (M) e
Estudo de coorte retrospectivo com avaliação dos laudos de exames US e blocos de parafina coletados de pacientes acompanhadas no pré-natal

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especializado com doença falciforme de 2011 a maio 2017. Para avaliação ecográfica, será utilizado como referência de normalidade de peso fetal
os dados do Fetal Growth Study (FGS), para comparação com parâmetros de normalidade, por idade gestacional.Para avaliação placentária, serão
utilizados os laudos de avaliação anatomopatológica disponíveis no prontuário médico.Para casos após 2016, teremos a possibilidade de utilizar
tecido fixado em bloco de parafina armazenado em Biobanco, com coleta sistemática de placenta (coleta de tecido) controlada através de
Procedimento Operacional Padrão (POP – Placenta Biobanco CAISM conforme anexo no projeto). Este material será avaliado por patologista
treinado para avaliação morfológica detalhada. O Biobanco do CAISM teve a aprovação de seu regimento em maio/2016 pelo Conselho Nacional de
Ética em Pesquisas, CONEP, e já se encontra instalado como estrutura institucional (registro CONEP B-056). As amostras são armazenadas
conforme normativas protocolares quanto à temperatura de armazenamento e estrutura de backup para evitar risco de perda. São coletadas
amostras apenas após assinatura do Termo de Consentimento Livre e Esclarecido (TCLE). O TCLE permite à mulher a escolha de: a cada nova
pesquisa realizada com o material biológico concedido e armazenado no Biobanco do CAISM do Hospital da Mulher Prof. Dr. José Aristodemo
Pinotti FCM/UNICAMP ser contatada para ler o Termo de Consentimento Livre e Esclarecido da nova pesquisa e decidir se permite ou não que a
amostra armazenada seja utilizada; ou que novas pesquisas podem ser realizadas com o material biológico concedido e armazenado no Biobanco
do CAISM do Hospital da Mulher Prof. Dr. José Aristodemo Pinotti FCM/UNICAMP sem a necessidade de aprovação para uso em cada uma delas.
Para o projeto em questão, somente os casos com esta autorização (sem a necessidade de aprovação para uso em cada uma delas) serão
selecionados.
Critério de inclusão:
- Gestantes com doença falciforme e Seguimento no Pré-Natal Especializado do CAISM- Parto no CAISM- ao menos 2 exames de US realizados no
CAISM

Continuação do Parecer: 2.308.740

Critério de Exclusão:
- parto em outra instituição
- não ter feito ao menos 2 exames de US na instituição.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar parâmetros ultrassonográficos, Dopplervelocimétricos e achados anatomopatológicos placentários nas gestantes com doença falciforme e correlacionar com resultados maternos e perinatais.

Objetivo Secundário:

1. Avaliar índice de pulsatilidade (IP) da artéria umbilical e cerebral média das gestantes com doença falciforme atendidas no ambulatório de pré-natal especializado, conforme idade gestacional, e comparar com a curva de Arduini e Rizzo de normalidade para parâmetros Dopplervelocimétricos (64).2. Avaliar o peso fetal estimado a partir de 28 semanas e comparar com a curva de normalidade de peso fetal do Fetal Growth Study para suspeita de RCF.3. Avaliar a presença de alterações morfológicas na placenta (maturidade, presença de fibrina no espaço interviloso, membrana sincicial, aglutinação vilositária, nós sincicials, hipoplasia de vilos distais, vilos avasculares, viloslte crônica, excesso de fibrina perivilosa, dismorfismo vilositário) e correlaciona-las com resultados maternos, perinatais e ultrassonográficos.

Avaliação dos Riscos e Benefícios:

Riscos:nenhum (deve ser modificado porque não há riscos previsível).

Benefícios:

correlação dos resultados de US com dados fetais poderão auxiliar no atendimento obstétrico de futuras gestantes com anemia falciforme, além de
auxiliar com protocolos de atendimento.

Comentários e Considerações sobre a Pesquisa:

Estudo de coorte retrospectivo com avaliação dos laudos de exames US e blocos de parafina coletados de pacientes acompanhadas no pré-natal especializado com doença falciforme de 2011 a maio 2017, com cerca de 41 pacientes com Anemia falciforme.

Considerações sobre os Termos de apresentação obrigatória:

Comprovante de vínculo; adequado

TCLE- pede dispensa e justifica: porque o estudo depende da revisão de dados de prontuários e

não haverá intervenções junto às pacientes, associado à dificuldade de localização e contato com as pacientes. Os casos que necessitarem de material do Biobanco, conforme descrição prévia, já terão TCLE assinado no momento da coleta da amostra (conforme Anexo no projeto) e somente aqueles com autorização para o uso do material em outras pesquisas sem necessidade de reconsentimento, serão utilizados.

Conclusões ou Pendências e Lista de Inadequações:

Mesmo sendo um número pequeno de pacientes e factível de convocação, aprovamos o protocolo de pesquisa, visto que as pacientes incluídas serão aquelas que aceitaram participar do Biobanco do CAISM e assinaram o TCLE do biobanco optando pela dispensa do reconsentimento.

Considerações Finais a critério do CEP:

- O sujeito de pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (quando aplicável).

- O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (quando aplicável).

- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando a aprovação do CEP para continuidade da pesquisa. Em caso de projetos do Grupo I ou II

apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.

- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

-Lembramos que segundo a Resolução 466/2012 , item XI.2 letra e, "cabe ao pesquisador apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento".

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_967798.pdf	12/08/2017 14:18:54		Aceito
Outros	Id_funcional.pdf	12/08/2017 14:18:31	Maria Laura Costa do Nascimento	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_doutorado_Camila_plataforma.pdf	07/08/2017 21:41:50	Maria Laura Costa do Nascimento	Aceito
Outros	Parecer_Circunstanciado.pdf	06/08/2017 21:25:07	Maria Laura Costa do Nascimento	Aceito
Folha de Rosto	Folha_de_rosto.pdf	06/08/2017 21:23:04	Maria Laura Costa do Nascimento	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 02 de Outubro de 2017

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador)

8.4 Anexo 4- Confirmação de submissão dos artigos científicos

8.4.1 – Artigo 1

The role of anemia in term and preterm pregnancies: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP)

Archives of Gynecology and Obstetrics

Dear Mrs Costa,

Thank you for submitting your manuscript,
"The role of anemia in term and preterm pregnancies: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP)", to Archives of Gynecology and Obstetrics

The submission id is: ARCH-D-21-01412
Please refer to this number in any future correspondence

During the review process, you can keep track of the status of your manuscript by accessing the following web site:

https://www.editorialmanager.com/arch/

8.4.2 – Artigo 2

Main complications during pregnancy and recommendations for adequate antenatal care in Sickle cell disease: a review of literature

Revista Brasileira de Ginecologia e Obstetrícia

03-Aug-2021

Dear Dr. Costa:

Your manuscript entitled "Main complications during pregnancy and recommendations for adequate antenatal care in Sickle cell disease: a review of literature" has been successfully submitted online and is presently being given full consideration for publication in the Revista Brasileira de Ginecologia e Obstetrícia.

Your manuscript ID is RBGO-2021-0295.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://mc04.manuscriptcentral.com/rbgo-scielo and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://mc04.manuscriptcentral.com/rbgo-scielo.

Thank you for submitting your manuscript to the Revista Brasileira de Ginecologia e Obstetrícia.

Sincerely,
Revista Brasileira de Ginecologia e Obstetrícia Editorial Office

8.4.3 – Artigo 3

High maternal perinatal morbidity and placental abnormalities in sickle cell disease pregnancies: a single-center retrospective analysis

Scientific Reports

Dear Dr Figueira,

Please note that you are listed as a co-author on the manuscript "High maternal perinatal morbidity and placental abnormalities in sickle cell disease pregnancies: a single-center retrospective analysis", which was submitted to Scientific Reports on 26 August 2021 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Peer Review Advisors
Scientific Reports

8.5 Anexo 5- Artigo publicado

Original Research *Highlight Article*

Different morphological and gene expression profile in placentas of the same sickle cell anemia patient in pregnancies of opposite outcomes

Letícia C Baptista[1,*], Camilla O Figueira[2,*], Bruno B Souza[1], Kleber Y Fertrin[3], Arthur Antolini[4], Fernando F Costa[5], Mônica B de Melo[1,*] and Maria Laura Costa[2,*]

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Corresponding author: Mônica B de Melo. Email: melomb@uol.com.br

Impact statement

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases. The rare opportunity to evaluate the same patient, with sickle cell anemia (SCA), in two different pregnancies, of opposite outcomes (one early onset severe pre-eclampsia (PE) and the other mostly non-complicated) can prove such concept. In addition, the comparison to other conditions of known placental and vascular/inflammatory involvement strengthens such findings. Our results suggest that the clinical association between SCA and PE can be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism may be important drivers of the pathophysiology of PE. Future studies using in a larger number of samples should confirm these findings and explore pathways involved in the pathophysiology of PE and its relationship with SCA.

Abstract

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases. Sickle cell disease (SCD) is a common inherited hemoglobin disorder characterized by chronic hemolytic anemia and vaso-occlusive crisis. SCD leads to higher morbidity and mortality, especially during pregnancy, with increased risk of preeclampsia (PE). To compare clinical findings, placental morphology, and gene expression in villous placental tissue using next generation sequencing. We included five cases. Two placentas from the same woman with homozygous SCD that had been pregnant twice and had different maternal and fetal outcomes (one early onset PE/eclampsia and a mostly non-complicated pregnancy); an early onset PE, a fetal growth restriction and a term, non-complicated pregnancy. Sixty-four differentially expressed genes were observed in the SCD+PE case, in comparison with the placenta from the SCD without PE, based on fold change. Among these genes, 59 were upregulated and 5 were downregulated. Enrichment analysis indicated two significant biological processes: response to copper ion (CYP1A1, AOC1, AQP1, and ATP5D) and triglyceride-rich lipoprotein particle clearance (GPIHBP1, APOC1, and APOE). The rare opportunity to evaluate the same patient in two different pregnancies, of opposing outcomes, and compare to other conditions of known placental and vascular/inflammatory involvement, may further the understanding of the pathophysiology of PE in SCD. Our results suggest that the clinical association between SCD and PE may be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism could be important drivers of PE pathophysiology.

Keywords: Placenta, sickle cell disease, gene expression, preeclampsia, and pregnancy

***Experimental Biology and Medicine* 2019; 244: 395–403. DOI: 10.1177/1535370219834305**

Introduction

Sickle cell disease (SCD) is one of the most common inherited diseases worldwide,[1] affecting around 300,000 newborns every year.[2–4] Birth rates of SCD vary according to the location; in the Americas, it is about 0.49 per 1000 live births.[5] The *HBB* Glu6Val genetic mutation causes the production of abnormal ß globin chains forming hemoglobin S

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(Hb6), and homozygosity for this mutation has the most severe clinical presentation.[1,2,6] HbS polymerizes when exposed to lower oxygen levels, making erythrocytes rigid and sickle shaped. These changes also cause increased cell adhesion to the endothelium, triggering its activation and the production of proinflammatory cytokines and chemokines.[7] Sickled red blood cells have a shorter lifespan, leading to chronic hemolytic anemia, and increased cell adhesiveness is the pathophysiological basis for the characteristic vaso-occlusive crises,[6,7] with higher morbidity[2,8,9] and mortality[8,10] with detrimental impact on quality of life.

SCD is especially challenging during pregnancy as it increases maternal and fetal morbidity and mortality.[2] Maternal complications include worsening anemia and a higher susceptibility to pain crises, acute chest syndrome, and infections.[1,11] SCD also doubles the risk of preeclampsia (PE) and increases the risk of eclampsia in homozygous patients by five times.[8] Fetal complications include higher risk of growth restriction, fetal demise, low birth weight, and prematurity.[8,10,12,13]

The placenta plays an important role in the severity of fetal complications, but the mechanisms are still not fully understood. Lower placental oxygen levels (due to anemia and sickling episodes) may lead to chronic inflammation and a hypoxic environment.[7] This proinflammatory environment could support the vaso-occlusive episodes and tissue necrosis,[14,15] which can further decrease the placental function and contribute to fetal growth restriction, PE and prematurity.[7]

The study presents a case of a patient with homozygous SCD that was pregnant twice and had different maternal and fetal outcomes. To further characterize the differences between her complicated and uncomplicated pregnancies, we compared clinical findings, placental morphology, and gene expression in placental tissue using next generation sequencing. The molecular findings were also compared with non-SCD cases without complications, with PE, and with fetal growth restriction (FGR). Our data emphasize the intra-individual heterogeneity of pregnancy in SCD, and suggest that studying placental abnormalities may help explain the some aspects pathophysiology of obstetric complication in SCD.

Materials and methods

Study group

This study was approved by the local Ethics Board (approval number 5404/2015). Pregnant women were recruited from the high-risk outpatient clinic and maternity of the University of Campinas (UNICAMP), and all samples and clinical data were collected upon written informed consent. This study included one woman with SCD (Hb6S), one woman with PE, one woman that delivered a baby with FGR, and one woman with non-complicated pregnancy as a control. All included cases delivered by cesarean section, without labor. Characteristics of the patients included in this study are shown in Table 1. The selected conditions were chosen due to their placental findings and pathophysiology of diseases related to endothelial and inflammatory state.

Hemoglobinopathy diagnosis (Hb6S) was confirmed by clinical data and hemoglobin electrophoresis (high performance liquid chromatography, Variant II, Bio-Rad, USA). PE was defined as a blood pressure higher than 140/90 mmHg, with proteinuria of more than 0.3 g in a 24-h sample.[16] FGR was defined as birth weight below the 10th percentile of that anticipated for the given gestational age.[17]

Placental tissue collection and RNA extraction

Placentas (stored at +4°C) were sampled within 3 h after caesarean section. Placental villous tissue samples were randomly selected from the central areas close to the umbilical cord, as previously described.[18] Before collection, the placentas were weighed and photographed. The samples were collected (≈200 mg) in three different regions avoiding areas of visible infarcts or calcifications; they were then separated into three equal size pieces and washed with phosphate saline buffer to remove residual

Table 1. Maternal and perinatal outcomes of the same sickel cell anemia patient in two different pregnancies.

	SCA 1st pregnancy	SCA 2nd pregnancy
Maternal age	19	21
First medical appointment	10 weeks	11 weeks
Hb level at first trimester	8.4 mg/dL	7.2 mg/dL
GA at hospital admission (weeks)/ medical condition	13/ pain crisis 17/ pain crisis 20/ respiratory infection 28/ acute chest syndrome/ worsening anemia 33/ fetal growth restriction	24/ pneumonia 33/ pain crisis 35/ pain crisis 36/ worsening anemia
Prophylactic blood transfusion*	No	Yes (28 weeks)
Mode of delivery	C-section	C-section
GA at delivery	34 weeks	36 weeks
Newborn weight	1910 g	2585 g
5-min apgar score	10	10
Neonatal outcome	Neonatal death	Alive
Postpartum contraceptive method	Oral contraceptive	Oral contraceptive

*Prophylactic blood transfusion became a routine in the prenatal care since September 2011 and aims to keep Hb level above 9.0 mg/dL to reduce maternal and fetal complications and reduce overall HbS.
SCA: sickle cell anemia; Hb: hemoglobin; GA: gestational age; g: grams.

maternal blood. Immediately after collection, the tissues were frozen in liquid nitrogen and stored at -80°C until the RNA extraction. All samples were collected by the same person and using identical protocol. Total RNA was extracted using TRIzol Reagent (Ambion), followed by the RNeasy mini kit (Qiagen). Purity level and concentration of isolated total RNA were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific), and RNA integrity was determined using the Bioanalyzer 2100 system (Agilent Technologies).

RNA sequencing

RNA-seq was performed on the five placenta samples included in the study. RNA sequencing (RNA-Seq) has been employed in placental villous tissue to describe the comprehensive transcriptome of two placentas, from different pregnancies (two years apart) from one woman with SCD. For comparison, placental villous tissue from one FGR, one preeclamptic, and one control pregnancy (without complications), all with HbAA genotype, was also sequenced. Library preparation and RNA-Seq were performed by Life Sciences Core Facility (LaCTAD). Complementary DNA libraries were prepared using Illumina TruSeq RNA Sample Preparation Kit according to the manufacturer's protocol (Illumina). Paired-end 100 bp high output sequencing was performed on Illumina HiSeq2500. The raw sequencing data were evaluated by FastQC version 0.11.5 for quality control. Reads were aligned to human genome assembly (GRCh38.88) using STAR 2.5.

Differential gene expression analysis

Differential gene expression analysis of the RNA-Seq data was performed with the R package DESeq2 and edgeR. The differentially expressed genes (DEGs) list was restricted to genes showing a fold change more than fourfold difference. Heatmaps were constructed through of a web tool, ClustVis,[19] using Pearson's correlation coefficient.

Histology

The placental samples were fixed in buffered formalin for 24 h, and further paraffin-embedded for light microscopy. Five-micrometer-thick histological sections were obtained, deparaffinized in xylene, rehydrated in a series of ethanol, and washed in phosphate-buffered saline (PBS), stained for hematoxylin and eosin (H&E) in order to provide morphology examination. Images were acquired using a Zeiss Axiophot2 microscope with a color Olympus DP72 digital camera using Olympus cellSens Standard 1.14 software.

Results

Case description

We present a unique opportunity to analyze placentas from a same patient, from pregnancies two years apart (Table 1). A 19-year-old Caucasian female with sickle cell anemia (SCA), previously on regular follow-up at the Hematology clinic with no indication for hydroxyurea or chronic transfusions. Her last blood transfusion had been over six months before she got pregnant. Her first admission was at 13 weeks for pain crisis. Additional admissions occurred, including acute chest syndrome/worsening anemia (Hb 5.0 g/dL). At 33 weeks' gestation, an ultrasound revealed a growth restricted fetus with oligohydramnios and the patient was readmitted for fetal surveillance. At 34 weeks, she began with headache and epigastric pain, associated with high blood pressure. Due to the diagnosis of PE with severe features, treatment with intravenous magnesium sulfate was started and a C-section was subsequently performed due to worsening symptoms. During anesthesia, the patient had a seizure (eclampsia). After delivery, she was admitted at the intensive care unit (ICU). The newborn weighed 1910 g, with a 5-min Apgar score of 10, but died of pneumonia at 24 days of age. She began combined oral contraceptives postpartum but returned to our high-risk antenatal care at 11 weeks' gestation two years later for another unplanned pregnancy (Table 1). Due to her history of eclampsia, aspirin and calcium supplementation were prescribed.

A chronic transfusion program was started at 28 weeks; however, she needed additional transfusions in the setting of pain crisis. The ultrasound showed adequate fetal growth throughout pregnancy. She gave birth to a healthy newborn weighing 2585 g at 36 weeks. C-section was indicated due to worsening anemia, an unfavorable cervix, and patient's choice not to undergo induction. Both mother and child were discharged on the third day postpartum.

The SCA patient (cases 1 and 2) and the other cases are also described in Table 2. Case 3 is a control; a term pregnancy with no complications. Case 4 is an early onset PE with no underlying disease, a primigravida with normal hemoglobin electrophoresis that underwent a C-section at 33 weeks' gestation due to severe features of PE. Number 5 is a case of FGR with no other background (no chronic disease and not a smoker) that delivered at 34 weeks' gestation, by C-section, due to fetal distress. Low birth weight was confirmed postpartum (1640 g).

Placental histology

In the gross examination of the placentas from the same patient with SCA, it is possible to observe the maternal (Figure 1(a) and (c)) and fetal (Figure 1(b) and (d)) faces of each placenta. Note the extensive microcalcifications (Figure 1(a)), and velamentous insertion of the umbilical cord of the first gestation placenta (Figure 1(b)), a prognostic adverse finding.

Histological examination showed that the first pregnancy placenta has more pronounced perivillous fibrin deposition, an expected feature in SCA placentas, and hypermature villi, an adaptation to chronic uteroplacental hypoxia, while the second gestation placenta showed abundant tertiary villi, and (Figure 2) sickle-cell erythrocytes in the intervillous space, a common feature in SCD.

Table 2. Socio-demographic characteristics, maternal and perinatal outcomes of cases included in this study.

Case number	Socio demographic characteristics	Obstetric history	Maternal outcomes	Fetal outcomes
1-SCA	Ethnicity: Non white	Parity: 0	Eclampsia	5-min Apgar score: 10
1st pregnancy	Maternal age (years): 19	GA at delivery (weeks): 34 Placental weight (g): 410		Birth weight (g): 1910 Neonatal ICU: yes
2-SCA	Ethnicity: Non white	Parity: 1	Uneventful	5-min Apgar score: 10
2nd pregnancy	Maternal age (years): 21	GA at delivery (weeks): 36 Placental weight (g): 540		Birth weight (g): 2585 Neonatal ICU: no
3-Control	Ethnicity: White	Parity: 1	Uneventful	5-min Apgar score: 10
	Maternal age (years): 21	GA at delivery (weeks): 38 Placental weight (g): 570		Birth weight (g): 3160 Neonatal ICU: no
4-PE	Ethnicity: White	Parity: 0	PE-severe features	5-min Apgar score: 8
	Maternal age (years): 20	GA at delivery (weeks): 33 Placental weight (g): 430		Birth weight (g): 2115 Neonatal ICU: no
5-FGR	Ethnicity: White	Parity: 0	Uneventful	5-min Apgar score: 9
	Maternal age (years): 37	GA at delivery (weeks): 35 Placental weight (g): 515		Birth weight (g): 1640 Neonatal ICU: yes

SCA: sickle cell anemia; PE: preeclampsia; FGR: fetal growth restriction; GA: gestational age; ND: not determined; ICU: intensive care unit; g: grams.

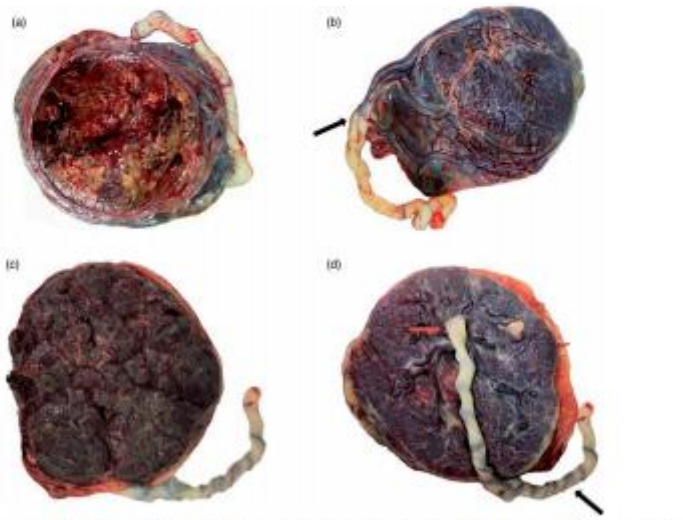

Figure 1. Macroscopic views of the placentas from the same patient with SCA in two different pregnancies. Placental gross examination from the same patient with SCA. Maternal (panel A) and fetal (panel B) sides of the placenta from a pregnancy complicated by severe preeclampsia. Maternal (panel C) and fetal (panel D) sides of the placenta from a pregnancy without preeclampsia. (a) extensive fibrin deposition and calcifications in about 40% of the maternal surface (low half of the figure), (b) umbilical cord with abnormal cord insertion (velamentous, black arrow), (c) well defined cotyledons, no gross abnormalities, and (d) umbilical cord with paracentral insertion (red arrow) and slight hypercoiling (black arrow). (A color version of this figure is available in the online journal.)

Overview of sequencing data of RNA-Seq analysis

After filtering, each individual transcriptome yielded a mean of 44 million 101-base pair paired-end reads (range: 26.17–58.86 million). GC content was approximately 48% for each sample. A minimum of 89% of the bases reached a Q score of 30 (Q30) and at least 68% of the sequence reads

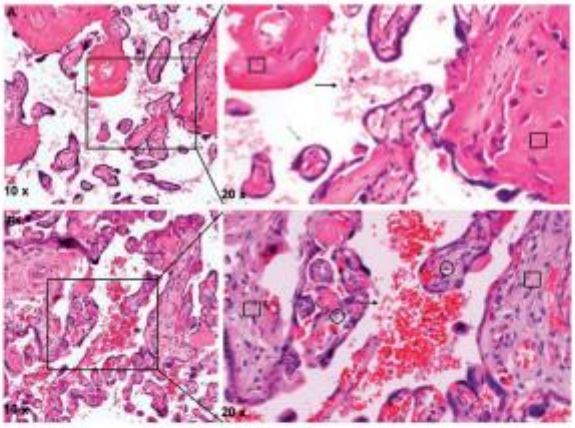

Figure 2. Placental histopathology assessed by H&E staining. Panel A represents the SCA patients first pregnancy and Panel B represents the second pregnancy, with the placenta seen in 10× magnification and further details in 20× magnification, with arrows in the most relevant findings. (a) First pregnancy placenta features: hypermature villi (gray arrow), hemolysis (black arrow), and accentuated perivillous fibrin deposition (square). (b) Second pregnancy placenta features: showing intermediate (square) and tertiary villi (circle) and sickle-cell erythrocytes (black arrow). (A color version of this figure is available in the online journal.)

Table 3. Differentially expressed genes identified by comparing the placenta from the same sickle cell anemia patient in her first pregnancy complicated with preeclampsia and fetal growth restriction (SCA+PE+FGR) with the placenta from her second pregnancy not complicated (SCA).

Gene	SCA+PE+FGR vs. SCA fold change
PSG9	−5.48
NAAT1	−4.85
CPXM2	−4.30
USPE	−4.15
PSG4	−4.12
LAMA3	4.00
HSPB1	4.00
FBXL15	4.00
ACTG2	4.00
AQP1	4.00
ATP5D	4.00
DKK1	4.00
GPBMBP1	4.00
IRF7	4.00
NDUFS7	4.00
PRG2	4.00
ROMO1	4.00
WDR18	4.00
KRT17	4.02
B3GNT7	4.02
PRKY	4.05
CAPG	4.05

(continued)

Table 3. Continued

Gene	SCA+PE+FGR vs. SCA fold change
OOF38	4.05
IL1R2	4.07
CDKN1C	4.08
KRT14	4.09
SCAND1	4.14
CST6	4.14
COMP	4.16
MRPL12	4.18
HN1	4.18
PPP1R14A	4.21
COX5B	4.26
FSTL3	4.28
NOS2	4.29
CLDN5	4.29
SOX18	4.33
GKN1	4.34
ALK8N7	4.34
CKB	4.35
TSPO	4.38
HLA-DRB5	4.39
NME3	4.44
SYT8	4.45
ADAMDEC1	4.51
ADC1	4.52
APOC1	4.53
APOE	4.55

(continued)

Table 3. Continued

Gene	SCA+PE+FGR vs. SCA fold change
VASN	4.55
HBM	4.59
CEBPD	4.60
PPDPF	4.67
B3GNT3	4.81
CRLF1	4.87
HLA-G	4.89
RAMP3	4.89
NOG	4.99
CCDC85B	5.04
SELENOM	5.11
CYP1A1	5.18
ASCL2	5.24
CFD	5.28
NOTUM	5.64
TAC3	5.84

were uniquely aligned to the human genome, with adequate read mapping evenness across the transcripts of all samples.

Identification of DEGs

Based on fold change (with a threshold above fourfold difference), 64 DEGs were identified from the comparison between the two placentas from the same patient, with and without complication by PE. Among those genes, 59 were upregulated and five were downregulated (Table 3). A graphic overview of the differential status of gene expressions is represented by the heatmap on Figure 3. The identified genes were analyzed according to Gene Ontology categories under biological process enrichment using WebGestalt (WEB-based GEne SeTAnaLysis Toolkit). The two most significant biological processes identified were response to copper (FDR < 0.006 for genes *CYP1A1*, *AOC1*, *AQP1*, and *ATP5D*) and triglyceride-rich lipoprotein particle clearance (FDR < 0.007 for genes *GPIHBP1*, *APOC1*, and *APOE*).

Discussion

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases.[20] The rare opportunity to evaluate the same patient, with SCD, in two different pregnancies, with opposite outcomes can prove such concept. And the comparison with other conditions of known placental involvement strengthens such findings.

Pregnancy in patients with SCA is known to have a higher risk for PE and FGR, complications associated with significant placental abnormalities. To the best of our knowledge, this is the first report of a rare opportunity to compare different placentas from the same patient in different pregnancies and outcomes.

The first pregnancy represents a typical situation of the high risk involving an SCA patient during pregnancy, with several hospital admissions, severe preterm PE, and eclampsia. In comparison, her second pregnancy was almost uneventful. We believe close surveillance, prophylaxis for PE, and programmed blood transfusions contributed significantly to a more favorable outcome, with no major complications. It appears important to decrease HbS during gestation in women with SCD, decreasing hemolysis and vaso-occlusion, and preventing their effects on the placenta, so blood transfusion may be of more benefit if started during the second trimester, or even earlier during the first trimester of gestation.[10]

The morphology of the placentas helps to exemplify the effect of PE on the placental architecture. In addition to this, the molecular analysis allowed us to uncover significant differences in gene expression with a paired analysis between placentas from the same patient, and to further explore the comparison with healthy control and placentas from patients with complicated pregnancies without SCA. We observed similarities in genetic expression among placentas with PE, FGR, and SCA with PE. These data agree with a previous study showing a common gene expression profile between PE and FGR.[21] Impaired deep placentation has been linked with a spectrum of complications during pregnancy including PE and FGR.[22] A possible interpretation might be that PE, and a subset of FGR are in fact manifestations of the same disorder caused by failure in trophoblast invasion leading to an abnormally shallow vasculature and impaired remodeling of the spiral arteries, and that the remaining differences depend on the degree of severity.[23]

Unlike in PE and FGR, placentas from women with SCD have seldom been studied. In our previous analysis,[10] we demonstrated that there is change in the expression of some genes associated with the inflammatory response, suggesting that the chronic inflammatory state in SCD can affect the placental physiology. Since both SCD and PE may impair the adequate development of the placenta and lead to maternal–fetal complications, the clinical association between SCD and PE may be explained by both diseases sharing a common genetic signature, with dysregulation of genes involved in inflammatory response and vascular endothelium dysfunction. In addition, increased free heme is a common factor among PE, FGR, and SCD and may be one of the responsible for changes in placental physiology.

Intravascular hemolysis, common in sickle cell patients, is a recurrent process that causes chronic inflammation. The release of inflammatory mediators and the recruitment of leukocytes and erythrocytes to the endothelium consequently result in endothelial dysfunction and vaso-occlusion, mainly in the microcirculation.[24] This process occurs most frequently during gestation, leading to the release of heme. On the other hand, in PE and FGR, the levels of free HbF and heme were found increased in the fetoplacental and maternal circulation.[25,26] The heme is considered a "damaging molecular pattern" (DAMP), in the circulation. DAMPs may activate multiple inflammatory pathways, including endothelial activation and injury.[27] According to Prophet *et al.*,[28] since SCD mothers already have ongoing processes that damage the maternal vascular endothelium, it could explain the likelihood of PE in patients with SCD. Besides being identified as a DAMP by the immune system, a study by Liu *et al.*[29] indicated

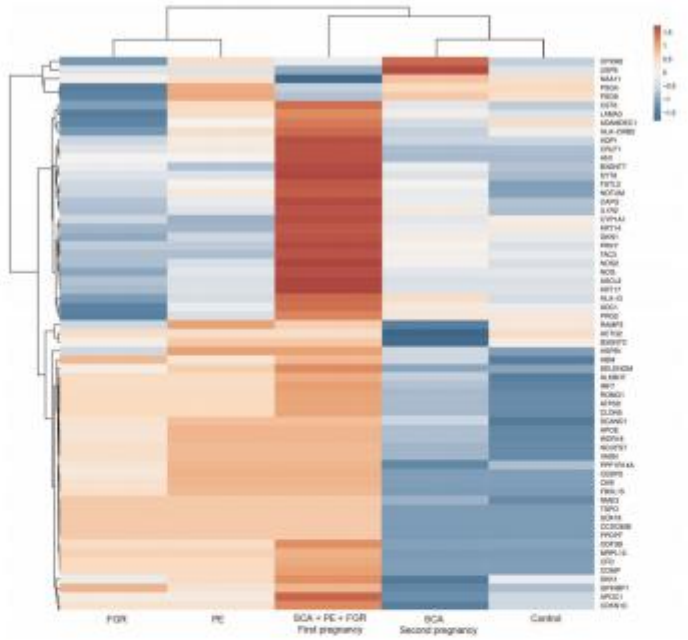

Figure 3. Heatmap generated from 64 genes with the greatest difference in expression between the placental samples from first pregnancy (SCA + PE + FGR) and second pregnancy (SCA). For comparison were also included placentas samples from patients with fetal growth restriction (FGR, *n* = 1), preeclampsia (PE, *n* = 1), and one healthy control. The figure represents hierarchically clustering the gene expression values of 64 genes. Up-regulated expressions are marked in red, down-regulations are colored in blue; white reflects no difference in expression levels. (A color version of this figure is available in the online journal.)

heme as an inhibitory agent of fusion of trophoblasts, that is, cytotrophoblasts cannot fuse to form the syncytiotrophoblasts. Because of this failure to differentiate cytotrophoblasts, the placenta may be impaired to perform nutrient and oxygen exchange, thus impairing fetal growth. Therefore, heme increase may be one of the factors involved in the higher risk PE in sickle cell patients. Nevertheless, not all patients with SCD will develop PE, and the case studied allowed us to investigate whether there is a genetic expression profile more associated with PE in the context of SCD. Nevertheless, not all patients with SCD will develop PE, and the case studied allowed us to investigate whether there is a genetic expression profile more associated with PE in the context of SCD.

Among the DEGs observed to be most up-regulated in the SCA placenta with PE in comparison with all other placentas, *HLA-G*, *TAC3*, *AOC1*, *B3GNT7*, *IL1R2*, and *PRG2* deserve particular attention, because these genes are specific to extravillous trophoblasts (EVTs), or highly expressed in these cells.[30] This suggests that the development of PE in SCA may be associated with an increase in the synthesis of EVT-derived proteins, and how this relates to impaired autophagy in EVT involved in poor placentation in PE is unclear.[31]

Increased expression of *HLA-DRB5* in SCA-associated PE is remarkable because the physiological lack of HLA major histocompatibility complex class II expression on trophoblasts is meant to prevent alloreactivity of maternal T cells against paternal antigens and allow successful tolerance. Aberrant expression of HLA-DR in PE has been very recently reported[32] and our results confirm that placental up-regulation of *HLA-DRB5* seems to be associated with

development of PE in SCA. This may be worthy of investigation as to what mechanisms elicit the expression of HLA-DR in the placenta that could explain the development of PE. Furthermore, there is evidence that associates certain HLA-DRB haplotypes with the appearance of either allo- or autoantibodies against red blood cell antigens in SCD patients, thus implying that abnormal HLA-DR expression may support the existence of a dysregulated immune system in SCD that could favor PE.[33]

Interestingly, five genes (*CPXM2, USP6, NAAT1, PSG4,* and *PSG9*) were downregulated in SCA combined with PE as well as in FGR. Of these genes, two are pregnancy-specific glycoproteins (PSGs) secreted by the placental syncytiotrophoblast.[34] Low PSG levels are associated with adverse pregnancy outcomes including FGR, PE, and spontaneous abortion, suggesting the importance of PSGs for a successful pregnancy.[35–37] To further elucidate the role of PSGs, Jones *et al.*[38] showed that PSG9 is important to the induction of immune tolerance during pregnancy. This suggests again that in pregnant patients with SCD, the immune tolerance may be decreased with a consequent attack on the placenta favoring the development of PE.

One of the most significant biological processes identified in association with PE included genes involved in the response to copper (*CYP1A1, AOC1, AQP1,* and *ATP5D*). This may relate to previous descriptions of the association of increased copper levels with occurrence and severity of PE[39] and with some populations of SCD patients,[40] although the exact mechanism by which levels of this metal increase in these diseases is unknown.

From these genes, *AQP1* encodes an aquaporin, which functions as a molecular water channel protein. Previous studies have also demonstrated *AQP1* expression in human and mice trophoblast cells[41] and this protein has been described to have roles in normal pregnancy, fetal growth, and homeostasis of amniotic fluid volume.[42] Dysregulated expression of *AQP1* is also associated with placental abnormalities[43] and PE.[44] An increased expression of *AQP1* in our case of SCA complicated with PE may be involved in the dysregulation of the amniotic fluid production and development of oligohydramnios.[45]

Up-regulation of genes involved in triglyceride-rich lipoprotein particles clearance were *GPIHBP1, APOC1,* and *APOE* and of the cytochrome P450 family 1 subfamily A member 1 (*CYP1A1*) gene suggest a defense mechanism of the trophoblasts against triglycerides in PE.[46,47] An association between hemolytic markers and triglyceride levels has been shown in SCD patients,[48] so the observed gene expression profile may recapitulate the severity of hemolysis and its effects on the placenta.

As a limitation, pairing of the patients was imperfect, with some significant differences in age and parity, although ethnicity, smoking status, and type of delivery were similar across samples. The placental samples used here included a mixture of cells, so we cannot determine which particular cells in the placenta are up- or downregulating the specific genes investigated. Of course it is a preliminary result and would be necessary to study a large number of patients, including some confirmatory studies with proteins. The strength of this study is the unique opportunity of exploring how clinical management during pregnancy can affect maternal/perinatal outcomes and placental development in an SCA patient.

Taken together, our results suggest that the clinical association between SCD and PE may be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism may be important drivers of the pathophysiology of PE. How and to what extent the clinical interventions currently being used to manage pregnant SCD patients affect these pathways remain to be determined. Future studies using placenta RNA-seq in a larger number of samples should confirm these findings and explore important pathways involved in the pathophysiology of PE and its relationship with SCD.

Authors' contributions: Conceived and designed the experiments: LCB, COF, MBM, and MLC. Performed the experiments: LCB, and AA. Analyzed the data: MLC, AA, LCB, COF, and BBS. Wrote the paper: KYF, LCB, COF, FFC, and MLC.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This study was supported by São Paulo Research Foundation (FAPESP) grant 2015/08330–5, grant 2014/00984–3 and the National Council for Scientific and Technological Development (CNPq) grant 409605/2016–6.

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(Received December 7, 2018, Accepted February 7, 2019)