UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

HEMOGLOBINA GLICADA (HbA1c) NO DIABETES *MELLITUS* GESTACIONAL

TESE DE DOUTORADO

PAULA BREITENBACH RENZ

PORTO ALEGRE, JANEIRO DE 2018.

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ORIENTADOR: PROFª. DRªJOÍZA LINS CAMARGO

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“A alegria não chega apenas no encontro do achado, mas faz parte do processo de busca.”

Paulo Freire

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LISTA DE ABREVIATURAS PARA A INTRODUÇÃO

ADA - *American Diabetes Association*

DM - Diabetes *mellitus*

DMG - Diabetes *mellitus* gestacional

DM1 - Diabetes *mellitus* tipo 1

DM 2 - Diabetes *mellitus* tipo 2

EBDG - Estudo Brasileiro de Diabetes Gestacional

ECDCDM - *Expert Committee on the Diagnosis and Classification of Diabetes Mellitus*

FPG - *Fast plasma glucose*

GJ - Glicemia de jejum

G1h - Glicemia após 1 hora

G2h - Glicemia após 2 horas

HbA1c – Hemoglobina glicada

IADPSG - *International Association of the Diabetes and Pregnancy Study Groups*

NIH - *National Institutes of Health*

OMS - Organização Mundial da Saúde

SBD - Sociedade Brasileira de Diabetes

TOTG - Teste Oral de Tolerância à Glicose.

RESUMO

De acordo com a Associação Americana de Diabetes (ADA), o Diabetes Melitus Gestacional (DMG) é definido como “Diabetes diagnosticado no segundo ou terceiro trimestre de gestação, tendo sido excluída a possibilidade de diabetes tipo 1 (DM1) ou tipo 2 (DM2)”. Devido ao elevado número de mulheres com DM2 não diagnosticado, é recomendável o rastreamento já na primeira visita pré-natal, utilizando critério diagnóstico padrão. A prevalência do diabetes na gravidez vem aumentando no mundo, a maioria DMG, mas também DM2 e DM1. Essa prevalência varia de acordo com a população estudada e o critério diagnóstico utilizado. Dentro dos riscos trazidos pela presença do diabetes não controlado na gestação estão o aborto espontâneo, anomalias fetais, pré-eclâmpsia, morte fetal, macrossomia, hipoglicemia neonatal, hiperbilirrubinemia neonatal entre outros. Além de prevenir os possíveis eventos adversos materno-fetais o diagnóstico de DMG na prática clínica também é importante devido ao risco para a mãe de apresentar diabetes no futuro.

Desde 1980, a OMS preconizava o uso do teste oral de tolerância à glicose (TOTG) após sobrecarga de 75g de glicose para o diagnóstico de DMG, utilizando os pontos de glicemia de jejum (GJ) e de glicemia 2 horas (G2h) após sobrecarga. Em 2010, a *International Association of the Diabetes and Pregnancy Study Groups* (IADPSG) recomendou que o critério diagnóstico para o DMG fosse baseado no estudo *Hyperglycemia and Adverse Pregnancy Outcomes* (HAPO), foram propostos então, novos pontos de corte para a GJ, glicemia de 1h (G1h) e G2h. Outro teste utilizado para o diagnóstico do diabetes *mellitus* (DM) desde 2010 é a hemoglobina glicada (HbA1c), também utilizada desde os anos 80 como uma ferramenta de avaliação do controle glicêmico em pacientes com DM. Em uma recente metanálise,

o teste HbA1c mostrou-se um teste acurado para a detecção de DMG em mulheres chinesas, indicando uma excelente acurácia do teste para diagnosticar DMG.

Com o objetivo de avaliar o uso do teste de HbA1c como teste diagnóstico e como teste preditor de complicações materno-fetais, realizamos um estudo em uma coorte de mulheres grávidas atendidas no Hospital de Clínicas de Porto Alegre (HCPA). Estudos mostram que a terapia para restabelecer os níveis de ferro nos pacientes pode levar à diminuição nos níveis de HbA1c, por isso avaliamos a possível interferência da suplementação de ferro durante a gestação nos níveis de HbA1c. Essa suplementação demonstrou não afetar os níveis de HbA1c e não ter impacto clínico na interpretação final dos resultados na ausência de anemia ou presença de anemia leve em nosso estudo. Quando realizamos uma revisão sistemática da literatura com metanálise, incluindo oito estudos, para avaliar a utilização da HbA1c no diagnóstico do DMG, nossos resultados mostraram que a partir do ponto de corte de HbA1c 5,8% tem-se especificidade suficiente para o diagnóstico do DMG. Quando avaliamos a associação da HbA1c, utilizado como teste diagnóstico para DMG, com desfechos materno-fetais, encontramos um aumento significativo na ocorrência de hipertensão gestacional relacionada com o aumento nos níveis de HbA1c.

ABSTRACT

According to the American Diabetes Association (ADA), Gestational Diabetes Mellitus (GDM) is defined as "Diabetes diagnosed in the second or third trimester of pregnancy, excluding the possibility of type 1 diabetes (DM1) or type 2 (DM2)". Due to the high number of women with undiagnosed DM2, screening at the first prenatal visit using standard diagnostic criteria is recommended. The prevalence of diabetes in pregnancy has been increasing in the world, mostly GDM, but also DM2 and DM1. This prevalence varies according to the population studied and the diagnostic criteria used. Among the risks brought by the presence of uncontrolled diabetes during pregnancy are spontaneous abortion, fetal anomalies, preeclampsia, fetal death, macrosomia, neonatal hypoglycemia, neonatal hyperbilirubinemia, among others. In addition to preventing potential maternal-fetal adverse events, the diagnosis of GDM in clinical practice is also important because of the risk for the mother to present diabetes in the future.

Since 1980, the WHO has recommended the use of the oral glucose tolerance test (OGTT) after overloading 75g of glucose for the GDM diagnosis, using fasting glucose (GJ) and glycemia 2 hours (G2h) after overload . In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended that the diagnostic criteria for GDM be based on the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, new cut-off points were then proposed for FPG, 1h (G1h) and G2h. Another test used for the diagnosis of diabetes mellitus (DM) since 2010 is glycated hemoglobin (HbA1c), has been used since the 1980s as a tool to assess glycemic control in patients with DM. In a recent meta-analysis, the HbA1c test proved to be an accurate test for the detection of GDM in Chinese women, indicating an excellent accuracy of the test to GDM diagnose.

In order to evaluate the use of the HbA1c test as a diagnostic test and as a predictor of maternal-fetal complications, a study was carried out in a cohort of pregnant women attending the Hospital de Clinicas de Porto Alegre (HCPA). Studies have shown that therapy to restore iron levels in patients may lead to a decrease in HbA1c levels, so we evaluated the possible interference of iron supplementation during pregnancy in HbA1c levels. This supplementation was found not to affect HbA1c levels and had no clinical impact on the final interpretation of the results in the absence of anemia or presence of mild anemia in our study. When we performed a systematic literature review with meta-analysis, including eight studies, to evaluate the use of HbA1c in the diagnosis of GDM, our results showed that from the cut-off point of HbA1c 5.8%, there is enough specificity for the diagnosis of GDM. When we evaluated the association of HbA1c, used as a diagnostic test for GDM, with maternal-fetal outcomes, we found a significant increase in the occurrence of gestational hypertension related to the increase in HbA1c levels.

SUMÁRIO

FORMATO DA TESE DE DOUTORADO

A presente tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, sendo apresentada através de uma breve fundamentação teórica e três manuscritos originais acerca do tema estudado:

1. Artigo original referente ao efeito do tratamento com ferro nos níveis de HbA1c durante a gravidez.

   "Effect of Iron Supplementation on HbA1c Levels in Pregnant Women with and without Anemia"

2. Artigo original referente à revisão sistemática com meta-análise de estudos de acurácia diagnóstica da HbA1c no DMG.

   "Diagnostic accuracy of glycated hemoglobin (HbA1c) for gestational diabetes mellitus (GDM): a systematic review and meta-analysis"

3. Artigo original referente a estudo longitudinal sobre a associação dos níveis de HbA1c na gravidez e os desfechos adversos materno-fetais.

   "Glycated hemoglobin (HbA1c) in pregnancy and adverse maternal-fetal outcomes"

1- INTRODUÇÃO

Definição de DMG

O Diabetes *mellitus* gestacional (DMG) durante muitos anos foi definido como “a hiperglicemia que é detectada pela primeira vez na gravidez, não excluindo a hiperglicemia pré-existente, podendo persistir após o parto” (ECDCDM 1997). Porém, de acordo com a Associação Americana de Diabetes (ADA) define-se como “Diabetes diagnosticado no segundo ou terceiro trimestre de gestação, tendo sido excluída a possibilidade de diabetes *mellitus* tipo1 (DM1) ou tipo2 (DM2)” (ADA 2017). Similar ao DM2, o DMG está associado tanto à resistência à insulina, quanto à diminuição da função das células beta-pancreáticas (Kuhl 2001).

Devido ao elevado número de mulheres com DM2 não diagnosticado, é recomendável o rastreamento já na primeira visita pré-natal, utilizando critério diagnóstico padrão **(Figura 1)**, caso positivo essa gestante será diagnosticada com diabetes pré-gestacional (DM2 ou, raramente DM1) (ADA 2017, SBD 2013/2014).

Prevalência de DMG

A prevalência do diabetes na gravidez vem aumentando no mundo, a maioria DMG, mas também DM2 e DM1 (ADA 2017). Essa prevalência varia de acordo com a população estudada e o critério diagnóstico utilizado, podendo aproximar-se de 18% das gestações quando utilizado o critério diagnóstico proposto pela *International Association of the Diabetes and Pregnancy Study Groups* (IADPSG) (Metzger 2010, ADA 2011). No Brasil, de acordo com dados do Estudo Brasileiro de Diabetes Gestacional (EBDG) aproximadamente 7,6% das gestações são

complicadas pela hiperglicemia gestacional (Schmidt 2000). Na Finlândia, 8,9% de DMG de acordo com o critério da ADA em 2009; na Áustria um estudo de coorte entre 2001 e 2004 identificou uma prevalência de DMG de 27,6%, de acordo com critérios da OMS então empregados; e na Itália, dados entre 1999 e 2003, identificaram uma prevalência de 7% de DMG, de acordo com critérios da ADA (Buckley 2012). Além disso, a prevalência do DMG ao redor do mundo vem aumentando juntamente com a prevalência da obesidade e do DM2, e da tendência ao adiamento da maternidade por parte das mulheres no mercado de trabalho (ADA 2012).

Fatores de risco

Os fatores de risco para DMG são: idade materna avançada, sobrepeso, obesidade ou ganho excessivo de peso na gravidez atual, deposição central excessiva de gordura corporal, história familiar de diabetes em parentes de primeiro grau, crescimento fetal excessivo, polidrâmnio, hipertensão ou pré-eclâmpsia na gravidez atual, antecedentes obstétricos de abortamentos de repetição, malformações, morte fetal ou neonatal, macrossomia ou DMG, síndrome de ovários policísticos e baixa estatura (menos de 1,5 m) (SBD 2017).

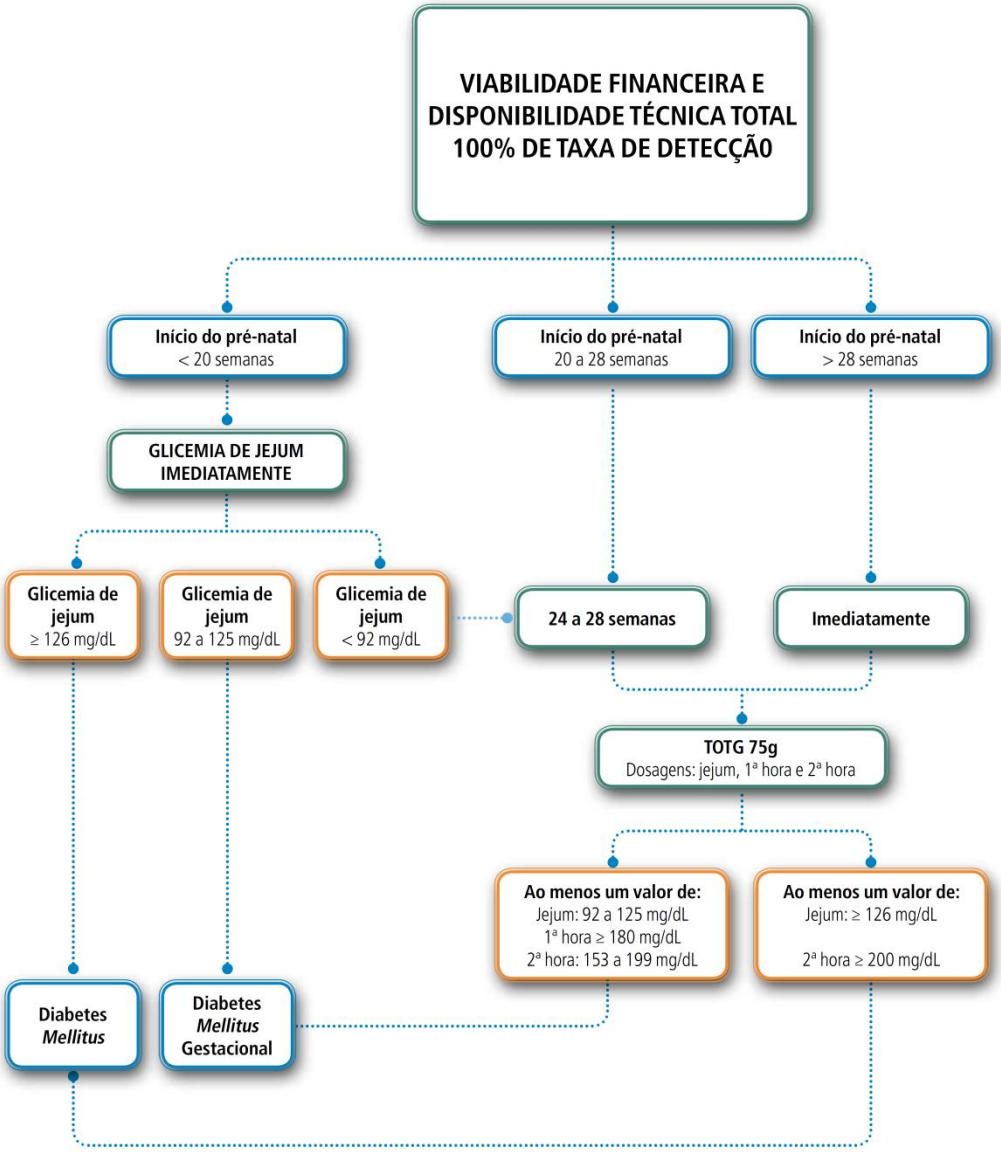

Figura 1. Rastreamento de DMG de acordo com a Organização Pan-Americana de Saúde (OPAS 2017) em regiões em situação de viabilidade financeira e disponibilidade técnica total. Teste Oral de tolerância à glicose - TOTG.

Importância clínica

Dentro dos riscos trazidos pela presença do diabetes não controlado na gestação estão o aborto espontâneo, anomalias fetais, pré-eclâmpsia, morte fetal, macrossomia, hipoglicemia neonatal, hiperbilirrubinemia neonatal entre outros (ADA 2017). Leng J et al., concluiu que o aumento da prevalência de DMG também se deu devido ao aumento de sobrepeso e idade das gestantes, além de relatar o risco elevado dessas gestantes desenvolverem DM2 e doenças cardiovasculares no futuro, mesmo negativando o diagnóstico no período pós-parto (Leng 2015). Isso pode estar relacionado ao déficit de secreção de insulina ou aumento na secreção desse hormônio. Também há evidências de maior risco de desenvolvimento de DM2 e obesidade nos filhos de mães com DMG (Boney 2005). Do ponto de vista obstétrico, há desfechos como doença hipertensiva, polihidramnio, parto prematuro, hipoglicemia, distócia de ombro, morte fetal e aumento no número de cesáreas devido a macrossomia (peso maior a 4Kg) (Metzger 2007).

O DMG traz riscos para a mãe e recém-nascido, mas nem todos os resultados são de importância clínica, porém em 2008, o estudo HAPO - *Hyperglycemia and Adverse Pregnancy Outcomes* que acompanhou aproximadamente 23mil mulheres com o objetivo de relacionar a hiperglicemia materna a eventos perinatais adversos, demonstrou que conforme aumenta o período gestacional, aumenta a ocorrência de eventos adversos, mesmo com intervalos antes considerados normais para gravidez, levando a uma reconsideração cuidadosa dos critérios diagnósticos para essa doença (Metzger 2008).

Além de prevenir os possíveis eventos adversos materno-fetais, o diagnóstico de DMG na prática clínica também é importante devido ao risco para a mãe de

apresentar diabetes no futuro (Yang 2002), mesmo com o desaparecimento da condição no período pós-parto.

Diagnóstico

- **Curva glicêmica e glicemia de jejum**

Há cinco décadas, O`Sullivan and Mahan propuseram um critério para diagnóstico de DMG, esses critérios foram adaptados para identificar gestantes em risco de desfechos adversos pré-natais (O'Sullivan 1964, O'Sullivan 1973).

Desde 1980, a OMS preconizava o uso do teste oral de tolerância à glicose (TOTG) após sobrecarga de 75g de glicose para o diagnóstico de DMG, utilizando os pontos de glicemia de jejum (GJ) e de glicemia 2 horas (G2h) após sobrecarga com os mesmos pontos de corte utilizados para diagnóstico de diabetes e tolerância diminuída à glicose na ausência de gravidez, inicialmente 140mg/dL e, após 1999, 126 mg/dL para a GJ, e 140 mg/dL para a G2h após sobrecarga de 75g de glicose (WHO 1999).

Na década de 90, foi realizado o Estudo Brasileiro de Diabetes Gestacional (EBDG), com cerca de seis mil gestantes (Schmidt 2001). Em 2002, de acordo com as recomendações da segunda reunião do Grupo de Trabalho em Diabetes e Gravidez, sugeriu-se o rastreamento universal do DMG, realizando-se teste de GJ a partir da 20ª semana de gestação como teste de rastreamento (sendo o ponto de corte de 85 mg/dL considerado positivo para rastreamento) com realização do TOTG com 75 g de glicose, tendo como critério diagnóstico do DMG a GJ ≥110 mg/dL ou G2h ≥140 mg/dL (Reichelt 2002).

Em 2010, a *International Association of the Diabetes and Pregnancy Study Groups* (IADPSG) recomendou que o critério diagnóstico para o DMG fosse baseado no estudo HAPO, foram propostos então, novos pontos de corte para o GJ, glicemia de 1h (G1h) e G2h, que são ≥92 mg/dL, ≥180 mg/dL e ≥153 mg/dL, respectivamente. Segundo esses novos critérios, ao menos um valor acima já leva ao diagnóstico de DMG (Metzger 2010). Esse critério foi adotado pela ADA em 2011 e é utilizado até hoje, embora também recomende a abordagem "de duas etapas", de acordo com o *American National Institutes of Health* (NIH 2013). O critério proposto pelo IADPSG também foi endossado pela OMS em 2013 (Metzger 2010, ADA 2011, WHO 2013). Porém esses estudos não são derivados de estudos em mulheres no primeiro trimestre de gestação por isso não se pode utilizá-los para esse fim nesse período gestacional, somente no período do segundo ou terceiro trimestre gestacional (ADA 2017). A Sociedade Brasileira de Diabetes (SBD) sugere a utilização dos novos critérios internacionais, pois são os únicos determinados por estudo que demonstra associação entre os valores da glicemia materna e os desfechos perinatais (4). O estudo de Leng J et al em 2015 observou aumento na prevalência de DMG quando utilizado o critério proposto pela IADPSG (Leng 2015). A **Tabela 1** descreve os critérios diagnósticos de acordo com as atuais recomendações da ADA: a curva glicêmica pode ser de uma etapa com 75g de glicose ou de duas etapas com 50g de glicose e posterior com 100g de glicose caso tenha sido positiva para 50g (ADA 2017).

Uma recente revisão sistemática da literatura foi realizada por Farrar et al. em 2017, avaliando os diferentes critérios para o diagnóstico de GDM com o objetivo de identificar os limiares que melhor predizem o aparecimento de eventos adversos maternos e fetais. No entanto, a revisão sistemática revelou que o uso de diferentes

critérios diagnósticos pelos estudos incluídos, os quais são arbitrários e muitas vezes baseados na opinião de especialistas, prejudicou a comparação de um método com outro, portanto os estudos nesta revisão não forneceram evidências suficientes para orientar a prática clínica e a política de saúde em relação à identificação de mulheres com GDM de acordo com um determinado critério diagnóstico (Farrar 2017).

Tabela 1. Critério diagnóstico para o DMG proposto pela A*merican diabetes Association* (ADA).

TOTG 75g glicose, 24-28 semanas de gestação (sem diagnóstico prévio de diabetes)*		
Glicemia de jejum	≥92 mg/dL (5,1 mmol/L)	
1h	≥180 mg/dL(10,0 mmol/L	
2h	≥153 mg/dL (8,5 mmol/L)	
Sobrecarga de 50g glicose após 1h ≥130,135 ou 140mg/dL, proceder TOTG 100g de glicose**		
	Carpenter/Coustan	NDDG
Glicemia de jejum	95 mg/dL (5,3 mmol/L)	105 mg/dL (5,8 mmol/L)
1h	180 mg/dL (10,0 mmol/L)	190 mg/dL (10,6 mmol/L)
2h	155 mg/dL (8,6 mmol/L)	165 mg/dL (9,2 mmol/L)
3h	140 mg/dL (7,8 mmol/L)	145 mg/dL (8,0 mmol/L)

*DMG= Ao menos um resultado positivo; **DMG= ao menos dois resultados positivos. National Diabetes Data Group (NDDG).

- **Novos marcadores**

Hemoglobina glicada (HbA1c)

A hemoglobina glicada (HbA1c) foi descoberta em 1958, é utilizada desde os anos 80 como uma ferramenta de avaliação do controle glicêmico em pacientes com DM, sua dosagem passou a ser cada vez mais empregada e aceita pela comunidade científica após 1993, depois de ter sido validada através dos dois estudos clínicos mais importantes sobre a avaliação do impacto do controle glicêmico sobre as complicações crônicas do DM: os estudos DCCT - *Diabetes Control and Complications Trial* (1993) e o UKPDS - *United Kingdom Prospective Diabetes Study* (1998), em pacientes DM1 e DM2, respectivamente (SBD 2017/2018).

Atualmente, a manutenção do nível de HbA1c ao redor de 7% é considerada como uma das principais metas de controle glicêmico para a maioria dos indivíduos com DM. Os dois estudos mencionados indicaram que as complicações crônicas começam a se desenvolver quando os níveis de HbA1c estão situados permanentemente acima de 7%. Metas terapêuticas mais ou menos rígidas para os valores de HbA1c, podem ser indicadas dependendo da presença de comorbidades e tipo de tratamento antidiabético adotado (SBD 2017/2018).

Em 2010, a ADA incluiu a HbA1c nos critérios para o diagnóstico do DM. O teste HbA1c é sugerido como um dos testes diagnóstico, e o ponto de corte de 6,5% é considerado como critério diagnóstico (ADA 2011). A OMS referendou esta recomendação em 2011 (Hanas 2010). No entanto, a HbA1c detecta uma população diferente de indivíduos, quando comparada com a GJ e TOTG, sugerindo que a

utilização de um conjunto de testes, ao invés de um teste isolado, pode ser uma estratégia mais eficiente para detectar DM (Cavagnolli 2011). Por outro lado, a conveniência para a realização do teste, como a ausência de necessidade de jejum, a menor variabilidade biológica e estabilidade da amostra após coleta podem suplantar esse aspecto (SBD 2017/2018). O diagnóstico de DMG não foi inserido nessas recomendações.

Na gestação, ocorre um aumento do volume sanguíneo total de 40 a 50%, porém o aumento do volume plasmático e da massa eritrocitária não é proporcional, provocando uma hemodiluição e redução dos indicadores hematológicos (hemoglobina e hematócrito), além de tempo de meia-vida dos eritrócitos, diminuído na segunda metade da gestação (Souza 2002). Alguns estudos sugerem que a anemia possa causar uma diminuição nos níveis de HbA1c (O'Connor 2012), porém em recente estudo observamos que isso depende do grau de anemia (Silva 2016). Alguns estudos relataram que a anemia por deficiência de ferro aumenta as concentrações de HbA1c em estados diabéticos e não diabéticos e que a terapia para restabelecer as reservas de ferro leva à diminuição da HbA1c (El-Agouza 2002, Coban 2004). Os níveis de HbA1c são mais baixos em mulheres grávidas quando comparadas a mulheres não grávidas. Devido a estes fatores, valores de referência diferenciados são recomendados na gravidez e sua interpretação deve ser realizada considerando estes fatores (Mosca 2006).

Em uma recente metanálise, a HbA1c mostrou-se um teste acurado para a detecção de DMG em mulheres chinesas, apresentando uma área sob a curva ROC (AUC) de 0,93, indicando uma excelente acurácia do teste para diagnosticar DMG. O ponto de corte de HbA1c ≥6,0% foi utilizado na maioria dos estudos (Tian 2013). Em um estudo realizado por nosso grupo sobre o papel da HbA1c como teste

diagnóstico para DMG mostrou que os níveis de HbA1c em gestantes sem DMG foram significativamente menores que os níveis de HbA1c nas gestantes com DMG, e que 38% dos casos de DMG foram diagnosticados usando o ponto de corte de HbA1c ≥5,8%, este ponto de corte apresentou especificidade suficiente para confirmar o diagnóstico. As gestantes foram então reclassificadas utilizando o ponto de corte de HbA1c ≥5,8%, e foi observado que o grupo com o diagnóstico de DMG pela HbA1c apresentou maior índice de massa corpórea (IMC), maior pressão sanguínea (sistólica e diastólica), maiores valores de glicemia e colesterol, além de apresentarem histórico prévio de DMG e histórico familiar de DM, em relação ao grupo sem o diagnóstico pela HbA1c, indicando que o teste HbA1c pode identificar mulheres que apresentam uma prevalência maior de indicadores de risco para desfechos adversos na gestação (Renz 2015).

Albumina glicada (AG)

A albumina glicada (AG) é um teste laboratorial que tem ganhado destaque como um marcador para o monitoramento glicêmico no DM (Kohzuma 2010, Koga 2010). A AG faz parte das frutosaminas, que são proteínas glicadas, porém, possui a vantagem de não sofrer influência da concentração de outras proteínas séricas, uma vez que é específica às taxas de glicação da albumina (Kohzuma 2002). Ainda, a AG reflete a glicemia de curto prazo, devido ao tempo de meia-vida da albumina, que é de aproximadamente três semanas, e não necessita de jejum para análise. Em comparação à HbA1c, a AG não é afetada pela presença de processos hemolíticos e de hemoglobinas anormais (Kim 2010). Em condições como anemia, gravidez, hiperglicemia pós-prandial e DM sob o uso de insulina, a AG parece ser um melhor marcador glicêmico (Koga 2010) e está especialmente indicada à

pacientes com DM submetidos à hemodiálise (Inaba 2007, Freedman 2010, Sany 2013). Recentes estudos com pacientes DM1 (Nathan 2014) e DM2 (Selvin 2014) têm reportado uma associação da AG com as complicações em longo prazo da doença.

A AG é formada em um período de aproximadamente 2 a 4 semanas (Koga 2014). Essa característica faz com que a AG seja mais fortemente correlacionada com as medidas contínuas de glicose, por ser mais sensível às rápidas alterações da glicemia, as quais não podem ser eficientemente identificadas somente com uma medida isolada de glicose plasmática (Kim 2010). A AG é também mais adequada para monitorar o início da terapia medicamentosa, tanto no DM1 quanto no DM2, ou até mesmo para controle do ajuste de dose e mudança de medicação, pois os seus níveis diminuem mais rapidamente do que a HbA1c em um tratamento intensivo. Desta forma, a AG pode ser um marcador glicêmico interessante durante o período gestacional, quando as variações glicêmicas ocorrem em poucas semanas e devem ser manejadas de acordo para evitar danos à mãe e ao bebê. Li et al. em 2016 publicou um estudo no qual mostrou associação dos níveis de AG com o peso do neonato ao nascer, sendo que o risco de peso ≥3,5 Kg ao nascer e macrossomia aumentou significativamente com níveis de AG ≥13.0% em 24-28 semanas e ≥12.0% em 36-38 semanas de gestação (Li 2016). A AG pode ser um marcador alternativo à A1c em muitas ocasiões, embora existam alguns estudos sobre a sua utilidade na detecção do DMG, são necessários mais estudos para verificar o melhor ponto de corte a ser empregado.

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3. JUSTIFICATIVA

A detecção cada vez mais precoce de alterações nos graus de glicemia que possam ocorrer durante a gestação só é possível com o rastreamento para DMG durante essa fase, evitando complicações maternas e fetais.

Atualmente, as medidas de glicemia, como GJ ou TOTG, são os testes laboratoriais de escolha para o diagnóstico.

A HbA1c recentemente foi recomendada como critério de primeira escolha para o diagnóstico de DM na ausência de gravidez. Nosso grupo avaliou 262 grávidas, no terceiro trimestre de gestação, através do teste oral de tolerância à glicose (teste de referência) e teste HbA1c, e mostrou que o teste HbA1c possui alta especificidade para o diagnóstico de DMG sendo o primeiro ponto de corte com especificidade (94,9%) suficiente para confirmar o diagnóstico o ponto de HbA1c ≥5,8%, diferente do valor recomendado pelas entidades internacionais na população em geral. O uso da HbA1c na gestação é controverso devido às mudanças fisiológicas características na gestação interferirem nos resultados da HbA1c, tornando sua interpretação difícil. Entretanto este teste laboratorial apresenta menor variação biológica, maior estabilidade pré-analítica e maior reprodutibilidade analítica em relação às medidas de glicemia. Também apresenta maior praticidade na coleta, devido não necessitar de jejum e ingestão de solução de glicose, tornando-se mais confortável e rápido para a detecção de DMG.

4. OBJETIVOS

Avaliar o uso do teste de HbA1c como teste diagnóstico e como teste preditor de complicações materno-fetais, em uma coorte de mulheres grávidas atendidas no ambulatório do HCPA.

OBJETIVOS ESPECÍFICOS

- Avaliar a possível interferência da suplementação de ferro durante a gestação nos níveis de HbA1c.

- Realizar revisão sistemática da literatura com metanálise para avaliar a utilização da HbA1c no diagnóstico do DMG.

- Avaliar a associação dos níveis de HbA1c com desfechos materno-fetais.

5. ARTIGO I

Effect of Iron Supplementation on HbA1c Levels in Pregnant Women with and without Anemia

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Effect of Iron Supplementation on HbA1c Levels in Pregnant Women with and without Anemia

Short Title: **Iron supplementation and HbA1c in pregnancy**

Paula Breitenbach Renz [1]

Mayana Kieling Hernandez [1]

Joíza Lins Camargo [1,2*]

[1] Graduate Program in Endocrinology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

[2] Endocrinology Department, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil

* Corresponding Author:

Joíza Lins Camargo, PhD

Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre.

Rua Ramiro Barcellos, 2350; Prédio 12, 4° andar, Porto Alegre, RS, 90035-903, Brazil.

E-mail address: jcamargo@hcpa.edu.br

ABSTRACT

Background: Iron deficiency anaemia has been associated with higher HbA1c levels. However, during and after iron supplementation there is a decrease in HbA1c results, causing a misinterpretation. Our aim was to analyse the effect of iron supplementation on HbA1c levels in nondiabetic pregnant women with and without anaemia.

Methods: Pregnant women in prenatal care, without gestational diabetes (GDM) or previous diabetes mellitus (DM) that performed an oral glucose tolerance test (OGTT) in the third trimester of pregnancy were invited to participate. Clinical and laboratorial analyses were performed, including standardized questionnaire, OGTT, full blood count and HbA1c.

Results: A total of 231 pregnant women without DM or GDM were included in the study. According to anaemia and/or iron supplementation, we divided women in: no iron and no anaemia – Group 1 (N=86); no iron and with anaemia – Group 2 (N=29); iron and no anaemia – Group 3 (N=87); iron and anaemia – Group 4 (N=29). There was statistically a significant, although no clinically relevant, difference between HbA1c values in pregnant women in Groups 1 and 4 [5.1 ±0.4% (32 ±4.4 mmol/mol) and 4.8 ±0.3% (29 ±3.3 mmol/mol), P <0.01; respectively]. HbA1c values in pregnant women in Groups 1, 2 and 3 were similar, independently of anaemia [5.1 ±0.4% (32 ±4.4 mmol/mol); 5.0 ±0.4% (31 ±4.4 mmol/mol) and 5.0 ±0.4% (31 ±4.4 mmol/mol); p>0.05; respectively].

Conclusions: Iron supplementation during pregnancy does not affect HbA1c levels and has no clinical impact in the final interpretation of results in the absence of anaemia or presence of mild anaemia. Interpreting HbA1c results in pregnant women during iron therapy and with moderate or severe anaemia still requires caution.

1. Introduction

Gestational diabetes mellitus (GDM) is a prevalent and potentially serious condition that may lead to adverse outcomes in both mothers and neonates [1]. It is associated with preeclampsia, increased caesarean rates, and macrosomia [2]. The detection and adequate treatment of this condition reduces the risks on the mothers as well on the babies [3,4]. Maternal glucose levels are continuously associated with adverse pregnancy outcomes without apparent threshold level, and the diagnostic and for treating levels of hyperglycaemia have been derived from consensus [2,5,6].

Habitually, the oral glucose tolerance test (OGTT) has been the diagnostic test of choice for diabetes in the general population [7]. The cut-off of HbA1c 6.5% (48 mmol/mol) was established for the diagnosis, and endorsed by the WHO in 2011 [7,8]. However, its use for the diagnosis of GDM has not been recommended yet by any current guidelines [1,7,9]. Recent results from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study showed that HbA1c measurements, similarly to glycaemia levels, were significantly associated with all adverse outcomes, and higher levels of maternal HbA1c were related to greater frequency of adverse outcomes [10]. In addition, a large cohort study in New Zealand [11] reported that HbA1c ≥5.9% (41mmol/mol) at the first antenatal visit identified all cases of GDM and was associated with twofold risks of congenital anomalies, preeclampsia, shoulder dystocia and a threefold risk of perinatal deaths. In a previous study [12], we evaluated the performance of HbA1c test to detect GDM in comparison to the traditional OGTT, and HbA1c value ≥5.8% (40mmol/mol) showed specificity of 94.9% in diagnosing GDM. Other studies have also highlighted the potential role of HbA1c in the diagnosis and management of GDM [13,14].

However, there are several other factors, besides than glycaemia, which can affect HbA1c results [15-17]. Traditionally, some diseases and pathological states, such as anaemia and haemoglobinopathies are considered potential factors that can significantly alter HbA1c results. Recently, we reported that iron deficiency anaemia (IDA) affects HbA1c results and this effect is dependent on anaemia degree [18]. These changes are statistically significant but they may not be clinically relevant and the presence of slight anaemia is likely to have a minor effect on HbA1c levels. It is also known that HbA1c is influenced by the life span of red blood cells [19]. Some studies reported that IDA increases concentrations of HbA1c in diabetic and nondiabetic states and that the therapy to re-establish iron stores leads to diminished HbA1c [20,21]. Indeed, iron and erythropoietin lead to both statistically and clinically significant fall in HbA1c levels without any change in glycaemic control in diabetic patients with chronic kidney disease [22]. In addition, HbA1c levels are elevated in late pregnancy because of iron deficiency in diabetic and nondiabetic women [23,24] and overestimates glycaemic control due to IDA in pregnant women with diabetes [24].

During pregnancy, haemoglobin concentrations change overtime, to accommodate the increasing maternal blood volume and the iron needs of the foetus [25]. Its concentration declines during the first trimester, reaching its lowest point in the second trimester and starts to rise again in the third trimester [26-28]. Blood dilution related to anaemia is frequently observed during pregnancy. Iron supplementation is generally recommended during pregnancy to meet the iron needs for both mother and baby [29] but it seems to have a small downward effect on HbA1c levels in pregnant nondiabetic women [30].

In this study, we analysed the effect of iron supplementation on HbA1c levels in nondiabetic pregnant women with and without anaemia.

1. Subjects and Methods

2.1. *Study Design*

This is a cross-sectional study that was carried out from October 2009 to November 2015 at the Endocrinology Division and Clinical Pathology Department of Hospital de Clínicas de Porto Alegre (HCPA).

2.2. *Study Participants*

Pregnant women in prenatal care, without previous DM, that performed OGTT test in the third trimester of pregnancy were invited to participate. The study design is depicted in **Figure 1.** All women signed an informed consent form and answered a standardized questionnaire. The study protocol was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (GPPG-HCPA), under protocol number 14-0579.

We excluded women with DM or GDM and those with conditions known to interfere with or lead to the misinterpretation of HbA1c results, such as chronic renal disease and/or presence of haemoglobin variants [17].

Anaemia was classified following WHO criteria [31], according to total Hb concentration, as mild anaemia (Hb ≥10 g/dL and <11 g/dL); moderate anaemia (Hb ≥7 g/dL and <10 g/dL) and severe anaemia (Hb <7 g/dL).

In Brazil, prophylactic iron supplementation for all pregnant women when they start prenatal visits regardless of gestational age is part of prenatal care. In this way, pregnant women during iron treatment according to the Brazilian Ministry of Health recommendation (65 mg of Iron once a day) for at least 2 months before the recruitment were enrolled in the group with iron supplementation to minimize differences regarding the iron supplementation period and dosage [32].

2.3. *Analytical Methods*

After an overnight fast, blood samples were drawn to determine HbA1c levels, blood cell counts and glucose concentrations. A full blood count was performed by flow cytometry and the hematimetric indices mean corpuscular volume (MCV); mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) were calculated (ABX Pentra DX 120, HORIBA, Kyoto, Japan). HbA1c was determined by a HPLC method (Variant II Turbo HbA1c, BioRad Laboratories, Hercules, CA, USA). This is a National Glycohemoglobin Standardization Program (NGSP) certified method and is aligned with the International Federation of Clinical Chemistry (IFCC) reference method (http://www.ngsp.org/ifcc.asp). Analytical inter assay coefficient of variation (CV) in our lab was <3.0%.

The OGTT was performed according to WHO 2013 recommendations [1]. GDM was diagnosed by ADA/WHO 2013 criteria (one out of those three following cut-off points: fasting plasma glycaemia (FPG) 5.1 mmol/L (92 mg/dL), 1 h glycaemia (G1h) 10.0 mmol/L (180 mg/dL) and 2 h glycaemia (G2h) 8.5 mmol/L (153 mg/dL) [7].

2.4. *Statistical Analysis*

Data were expressed as mean and SD for normally distributed variables, and as median (interquartile range) for non-Gaussian variables. The Shapiro–Wilk test was applied to verify the normality of results. One way ANOVA and Mann–Whitney U testwere used as appropriate. Differences greater than the total error allowable for HbA1c (TE_a = 6%) were considered clinically relevant [33]. Considering the reference range for non-diabetic pregnant women in the literature [26-28], a power of 80% at a significance level of 5%, a minimum of 23 women in each group would be necessary to detect a relative clinically relevant difference of ±6% on HbA1c levels (http://clincalc.com/Stats/SampleSize.aspx). All data were analysed with IBM SPSS software for Windows, version 19.0 (Statistical Package for Social Sciences—Professional Statistics, IBM Corp, Armonk, USA). A significance level of 5% was adopted.

3. Results

A total of 231 pregnant women without DM or GDM were included in the study. All of them were in the third trimester of gestation (gestational age = 27 ± 4.4 weeks) with ages between 18 and 46 years. Of those, 115 were without iron supplementation (no iron) and 116 were during iron supplementation (iron). Anaemia was present in 29 pregnant women with iron supplementation and in 29 women without iron supplementation. According to the presence of anaemia and/or iron supplementation, they were divided into 4 groups: no iron and no anaemia – Group 1; no iron and with anaemia – Group 2; iron and no anaemia – Group 3; iron and anaemia – Group 4.

Table 1 depicts the clinical and laboratory characteristics of all patients. There was no statistic difference in body mass index (BMI), FPG, and G1h e G2h between groups. As expected, total haemoglobin (Hb) and haematocrit were statistically different between women with and without anaemia, independently of iron supplementation [12.1 g/dL (±0.8); 10.3 g/dL (±0.5); 11.9 g/dL (±0.7); 10.1 g/dL (±0.6) for Hb and 35.8% (±2.2); 31.2% (±1.6); 35.3% (±1.9); 30.1 (±1.9) for haematocrit, in groups 1, 2, 3, and 4 respectively]. There was no difference in MCV and MCHC values among groups. Women with anaemia and iron supplementation showed higher values for RDW than women without anaemia.

There were statistically significant differences between HbA1c values in pregnant women without iron supplementation and without anaemia and the HbA1c values from pregnant women during iron supplementation and with anaemia [5.1 ±0.4% (32 ±4.4 mmol/mol) and 4.8 ±0.3% (29 ±3.3 mmol/mol), P <0.01; for group 1 and 4, respectively]. HbA1c values in pregnant women during iron supplementation but without anaemia were similar to those in pregnant women without iron

supplementation, independently of the presence of anaemia [5.1 ±0.4% (32 ±4.4 mmol/mol); 5.0 ±0.4% (31 ±4.4 mmol/mol) and 5.0 ±0.4% (31 ±4.4 mmol/mol); p>0.05; for groups 1, 2, and 3 respectively].

HbA1c values for all groups were shown in **Figure 2**.

Table 1 Clinical and laboratory characteristics of all pregnant women participating in this study.

	No Iron Supplementation		Iron Supplementation	
	Without anaemia Group 1	**With anaemia Group 2**	**Without anaemia Group 3**	**With anaemia Group 4**
N	86	29	87	29
Age (years)	28 (±6.8)	29 (±6.4)	28 (±6.3)	29 (±5.7)
Gestational age (weeks)	26.3 (±5.2)	27.3 (±4.1)	27.1 (±4.1)	26.1 (±3.3)
BMI (Kg/m2)	29.7 (±6.3)	29.4 (±5.2)	28.5 (±5.2)	28.2 (±5.0)
Haematocrit (%)	35.8 (±2.2) [a]	31.2 (±1.6) [a,b]	35.3 (±1.9) [b]	30.1 (±1.9) [a,b]
Hb (g/dL)	12.1 (±0.8) [a]	10.3 (±0.5) [a,b]	11.9 (±0.7) [b]	10.1 (±0.6) [a,b]
MCV (fL)	89.2 (±4.4)	87.1 (±4.5)	88.5 (±5.7)	90.7 (±7.5)
MCHC (g/dL)	33.8 (±1.1)	33.1 (±1.2)	33.8 (±1.0)	33.5 (±1.0)
RDW (%)	13.6 (±0.9) [a]	13.8 (±1.4)	13.6 (±0.9) [b]	14.5 (±1.9) [a,b]
FPG (mg/dL)	79.8 (±5.3)	80.8 (±5.1)	79.1 (±6.0)	77.7 (±6.8)
G1h (mg/dL)	118.7 (±23.6)	109.4 (±22.8)	124.3 (±26.9)	114.2 (±31.5)
G2h (mg/dL)	110.4 (±22.7)	105.0 (±16.6)	107.6 (±20.6)	103.8 (±22.8)
HbA1c (%)	5.1 (±0.4) [a]	5.0 (±0.4)	5.0 (±0.4)	4.8 (±0.3) [a]
(mmol/mol)	32 (±4.4) [a]	31 (±4.4)	31 (±4.4)	29 (±3.3) [a]

Same letter within a row indicates difference between groups (P <0.05, by One-way ANOVA). BMI - body Mass index; Hb - haemoglobin; MCV - mean corpuscular volume; MCHC - mean corpuscular haemoglobin concentration; RDW - red cell distribution width; FPG - fasting plasma glucose; G1h - glucose 1h after OGTT; G2h - glucose 2h after OGTT.

4. Discussion

This study investigated the effect of iron supplementation on HbA1c levels in pregnant women without diabetes, with and without anaemia. Our results showed a small fall in HbA1c levels in pregnant women during iron supplementation and with anaemia when compared to pregnant women without iron supplementation and without anaemia. Although significant, this difference is not clinically relevant. We also observed that pregnant women without iron supplementation, showed similar HbA1c values independently of the presence of anaemia.

The increasing effect of iron deficiency on HbA1c values and the fall in its levels after treatment with iron in individuals with and without diabetes were already reported [19-24,34]. Also, some studies related absence of association of HbA1c level with ferritin, vitamin B12, and folic acid in elderly nondiabetic subjects [35] or a small, clinically irrelevant, decrease HbA1c values in adults [15,18] supporting the idea that HbA1c levels are independent of anaemia or nutritional factors associated with anaemia.

However, data of these effects on HbA1c levels during the pregnancy are scarce. In fact, HbA1c levels are lower in pregnant women than in the general population [12,26-28]. During pregnancy, HbA1c decreased in the second trimester and increased in the third trimester. This effect is attributed either to the physiological changes during pregnancy and/or the involvement of iron-deficiency anaemia [23,24,28]. In our study, pregnant women without DM or DMG showed HbA1c values between 4.2% and 5.9%, similar to those reported in earlier studies and lower than the values observed in the general population [12,26-28].

In fact, traditionally, pregnancy is considered an inherent limitation to the use of HbA1c, aside from other factors, such as anaemia, uraemia and presence of variant haemoglobin that may falsely increase, or decrease HbA1c levels independent of glycaemia [7,15]. Its use is not recommended to diagnose GDM by any guidelines, however, it is recommended to rule out pre-existent type 2 DM in the first trimester of gestation [1,7,9].

In addition, throughout pregnancy, women need iron to meet their own desirable iron levels as well as those of the developing baby. Iron supplementation has been the preferred intervention to improve iron stores and prevent anaemia among pregnant women, and it is associated with a reduced risk of anaemia and iron deficiency during pregnancy [36]. Up to date, only one study analysed the effect of iron supplementation on HbA1c levels in pregnant women and showed no effect on HbA1c values after 3 months of iron-supplementation [30].

Our study, in a cross-sectional design, showed that pregnant women during iron supplementation and without anaemia presented HbA1c values similar to those of women without iron supplementation and without anaemia. In addition, our results are in agreement with previous observations by our group that iron deficiency anaemia and/or iron deficiency has a minor effect on HbA1c levels in the general population [15] and this effect is dependent of degree of anaemia [18]. It is important to point out that the pregnant women who took part in our study presented only mild anaemia with haemoglobin levels around 10 g/dL [31] which contributed to absence of effect on HbA1c values in anaemic women without iron supplementation and to a small decline on HbA1c values in anaemic women during iron supplementation.

Iron supplementation during the pregnancy may not be a limitation to the use of HbA1c in the diagnosis of GDM or DM. It might also not be a limiting factor during the monitoring of these conditions through all pregnancy period. However, clinicians should be aware that HbA1c values are different in pregnant women.

4.1. *Strengths/Weakness*

This research has several strengths. One high point of this study is that we analysed the effect of iron supplementation on HbA1c levels during pregnancy in women with and without anaemia in the absence of DM or GDM to minimize as much as possible the HbA1c variability. Also, all conditions that may affect and mask HbA1c results were excluded. Moreover, HbA1c was measured by a method worldwide commonly used, standardized and traceable to IFCC and NGSP, respectively. In addition, a post-hoc analysis indicated that our study has 80% power to detect an effect size of 0.18 absolute HbA1c units (http://www.sample-size.net/means-effect-size/), a difference much lower than the TEa for HbA1c.This study also presents some weaknesses. We were not able to measure blood iron metabolism indexes such as ferritin, transferrin and iron binding capacity. However, these parameters are of limited usefulness during pregnancy, as their concentrations may be altered even in the absence of iron deficiency [31]. Because of the small sample size in the anaemia groups was not possible to stratify the data by the degree of anaemia, however the majority of patients presented mild anaemia, as reported early by our group [18] mild anaemia has a minor, and not clinically relevant, effect on HbA1c levels. Furthermore, the cross-sectional design of this study does not allow us to investigate causality. Finally, it should be mentioned that this study was performed in a group of pregnant women without DM or GDM and the degree of

these variations may be or may not be clinically relevant in the presence of hyperglycaemia.

4.2. Conclusions

Our results indicate that iron supplementation during pregnancy does not affect the results of HbA1c and does not have a clinical impact in the final interpretation of the results in the absence of anaemia or presence of mild anaemia. Interpreting HbA1c results in pregnant women during iron therapy and with moderate or severe anaemia still requires caution.

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Contribution Statement

Research idea and study design: PBR and JLC; data acquisition: PBR and MHK; data analysis/interpretation: PBR and JLC; statistical analysis: PBR and JLC; wrote the manuscript: PBR and JCL. JLC takes responsibility that this study has been

reported honestly, accurately, and transparently; that no important aspects of the study have been omitted.

Disclosure Statement

The authors declare no conflicts of interest to this work.

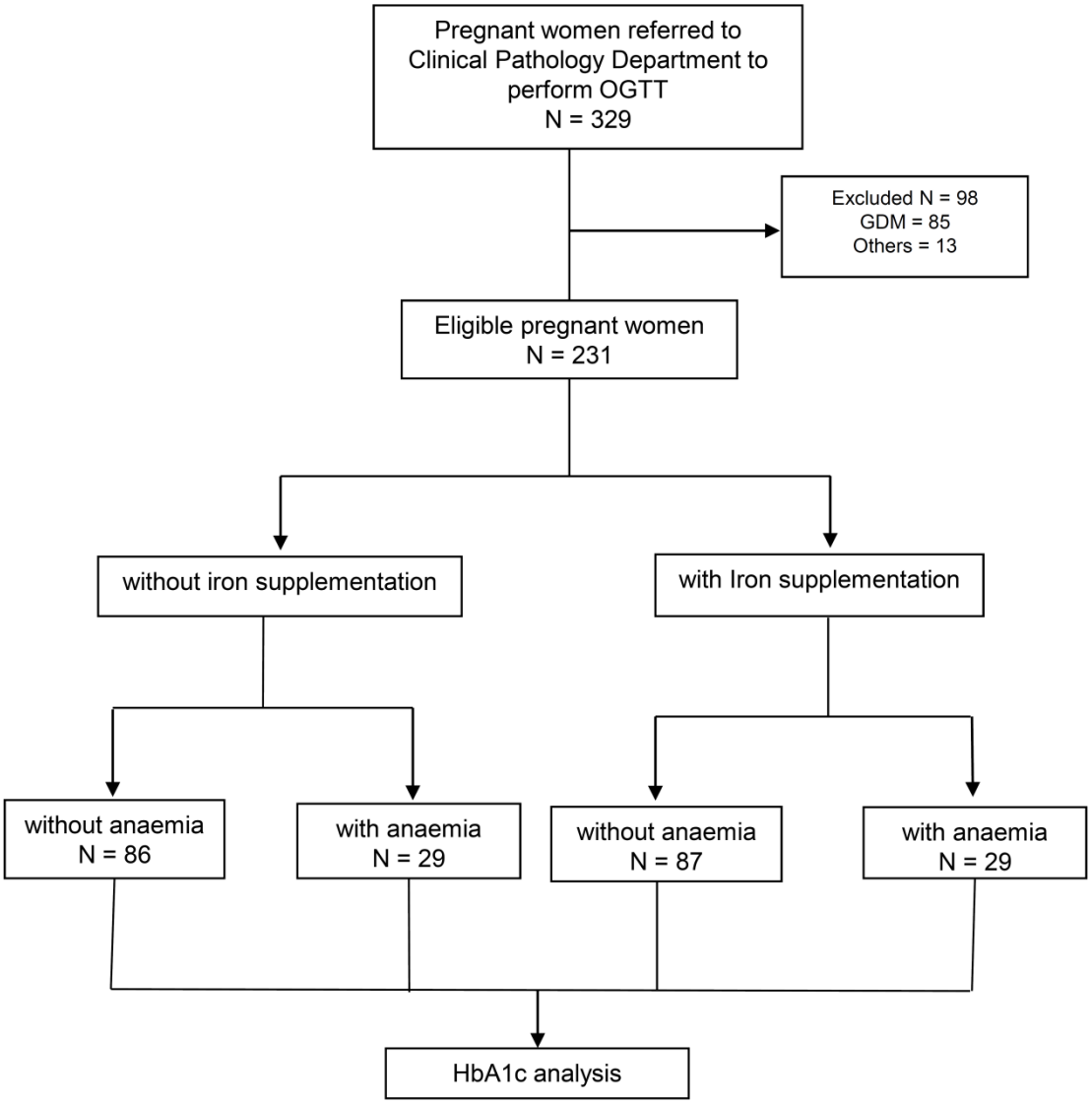

Figure 1 – Study design.

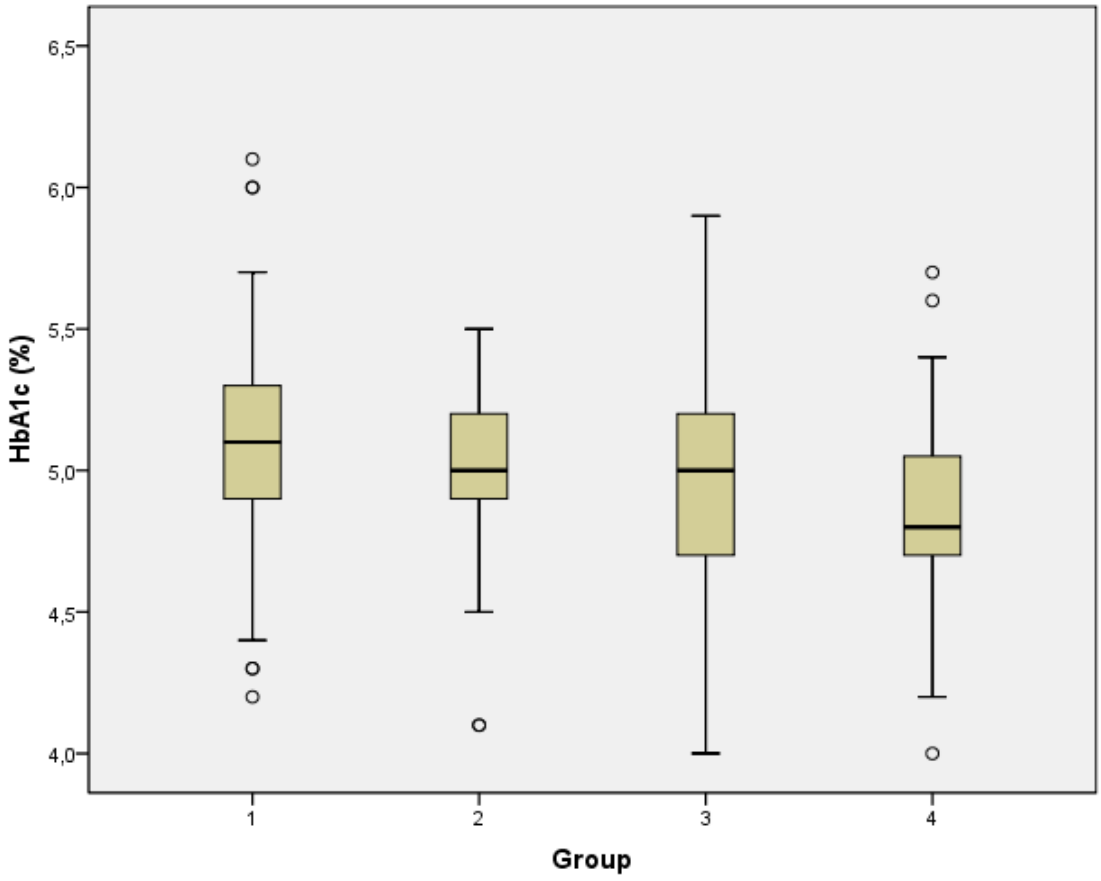

Figure 2 – Distribution of HbA1c values in pregnant women without iron supplementation and without anaemia (1); pregnant women without iron supplementation and with anaemia (2); pregnant women with iron supplementation and without anaemia (3); and pregnant women with iron supplementation and with anaemia (4). Each box shows the median, quartiles, and extreme values within group, one-way ANOVA used to compare groups.

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6. ARTIGO II

Diagnostic accuracy of glycated hemoglobin (HbA1c) for gestational diabetes mellitus (GDM): a systematic review and meta-analysis

Diagnostic accuracy of glycated hemoglobin (HbA1c) for gestational diabetes mellitus (GDM): a systematic review and meta-analysis

Paula Breitenbach Renz [1]
Fernando Chimela Chume [1,2]
João Rodolfo Teló Timm [1]
Ana Laura Pimentel [1]
Joíza Lins Camargo [1,3*]

[1] Graduate Program in Endocrinology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[2] Universidade Zambeze, Beira, Mozambique
[3] Experimental Research Centre, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil

* Corresponding Author: jcamargo@hcpa.edu.br

ABSTRACT

Background: Studies have shown the potential role of glycated hemoglobin (HbA1c) in the diagnosis and management of gestational diabetes mellitus (GDM). We conducted a systematic review and meta-analysis to establish the overall accuracy of HbA1c for the diagnosis of GDM. **Methods:** We searched MEDLINE, EMBASE and SCOPUS up to December 2017. Studies carried out with pregnant women without previous diabetes that assessed the performance of HbA1c (index test) compared to 75g OGTT (reference test) for the diagnosis of GDM, that measured HbA1c by standardized methods and presented data necessary for drawing 2x2 tables were included. **Results:** This meta-analysis included 8 studies, totaling 6,406 pregnant women, of those 1,044 with GDM. The diagnostic accuracy of HbA1c was reported at different thresholds ranging from 5.4% to 6.0%, and the area under the curve (AUC) was 0.825 (95% CI 0.751 – 0.899), indicating a good level of overall accuracy. The pooled sensitivities and specificities were 50.3% (95% CI 24.8% - 75.7%) and 83.7% (95% CI 67.5% to 92.7%); 24.7% (95% CI 10.3% - 48.5%) and 95.5% (95% CI 85.7% to 98.7%); 10.8% (95% CI 5.7% - 19.41%) and 98.7% (95% CI 96.2% to 99.5%);12.9% (95% CI 5.5% - 27.5%) and 98.7% (95% CI 97.6% to 99.3%), for the cutoffs of 5.4%, 5.7%, 5.8% and 6.0%, respectively. **Conclusion:** HbA1c test present high specificity but low sensitivity regardless of the threshold used to diagnose GDM. These findings point out to the usefulness of HbA1c as a rule-in test that should be used in association with other standard diagnostic tests for GDM diagnosis.

Keywords: diagnosis, HbA1c, gestational diabetes, meta-analysis.

INTRODUCTION

According to the American Diabetes Association (ADA), gestational diabetes mellitus (GDM) is "diabetes that is first diagnosed in the second or third trimester of pregnancy, that excludes the possibility of pre-existing type 1 or type 2 diabetes" (1). This disease is a prevalent and potentially serious condition that may lead to adverse outcomes in both mothers and neonates (2). It is associated with preeclampsia, increased caesarean rates, and macrosomia (3). The detection and adequate treatment of this condition reduce the risks for mothers as well as for babies (3,4,5).

The oral glucose tolerance test (OGTT) has been the diagnostic test of choice for diabetes mellitus (DM) in the general population (1). In the last decades, the diagnostic criteria for GDM have been controversial and a range of recommendations and guidelines to identify women with GDM have been proposed (1, 2, 6-9).

Up to 2013, the World Health Organization (WHO) recommended that the GDM diagnosis should be based on the same criteria used for non-pregnant adults using the 2h 75g OGTT, the cutoffs fasting glucose ≥7mmol/L or 2h glucose ≥7.8mmol/L were diagnostic for GDM (2). The UK National Institute for Health and Care Excellence (NICE) recommendations (9) are based on these criteria; however, they recommended a lower cutoff for fasting glucose ≥5.6 mmol/L. More recently, the International Association of the Diabetes in Pregnancy Study Group (IADPSG), after the results of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, a cohort study with about 25,000 pregnant women, recommended a new diagnostic criterion for GDM based also on 2h 75g OGTT but with lowered thresholds (fasting glucose ≥5.1mmol/L; 1h glucose ≥10.0 mmol/L; 2h glucose ≥8.5 mmol/L), GDM is present if one or more results are altered (10-12). Since 2013, WHO has adopted

these same IADSPG criteria (2). According to ADA, GDM diagnosis can be performed by the one-step 2h 75g OGTT using the same threshold diagnostic criteria of IADPSG or the two-step strategy with a 1h 50g OGTTT screen followed by a 3h 100g OGTT for those who screen positive (1).

Although OGTT is recommended as the diagnostic test for GDM by international organizations, it requires at least 8h fasting, an extensive patient preparation, lacks reproducibility, it is time-consuming and uncomfortable for pregnant women (7).

HbA1c test has been used in clinical practice for monitoring patients with DM since early 80´s (13), but its use in diagnosis was established only in 2010 (14). The cut-off of HbA1c 6.5% (48 mmol/mol) is recommended for DM diagnosis in the general population (Expert Committee 2010), and this cutoff is endorsed by ADA and WHO (10, 15). However, its use for the diagnosis of GDM has not yet been recommended by any current guidelines (1, 2, 7, 10). Results from the HAPO study showed that HbA1c values, similar to glycemia levels, were significantly associated with all adverse outcomes, and higher levels of maternal HbA1c were related to greater frequency of adverse outcomes (6). HbA1c test would be more receptive to this group of patients because of its convenience when compared to OGTT. However, due to some physiological and analytical factors that might interfere with HbA1c results it has not yet been included as a diagnostic tool for GDM (1, 16, 17).

Some studies have evaluated the diagnostic accuracy of HbA1c in DMG (18-22). In a recent meta-analysis with 2,812 patients and 5,918 controls, that measured HbA1c in Chinese pregnant women, showed that this test is a useful diagnostic tool for confirming GDM (18). A large cohort study in New Zealand reported that HbA1c ≥5.9% (41mmol/mol) at the first antenatal visit identified all cases of GDM and was

associated with a twofold risk of congenital anomalies, preeclampsia, shoulder dystocia and a threefold risk of perinatal deaths (19). We also showed that HbA1c levels may be a useful diagnostic tool for GDM in Brazilian pregnant women, the HbA1c cut-off point of 5.8% (40 mmol/mol) was able to diagnose 38% of GDM cases by OGTT and also 5% of pregnant women classified as GDM-negative by the OGTT were identified according to the HbA1c test (20). Other studies have also highlighted the potential role of HbA1c in the diagnosis and management of GDM (21, 22).

In this study we carried out a systematic review and meta-analysis to determine the diagnostic accuracy of HbA1c test in the diagnosis of GDM in different populations of pregnant women.

MATERIALS AND METHODS

Search strategy

This meta-analysis is in agreement to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement (23) and in according to the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (24). We searched PubMed (MEDLINE), Embase and SCOPUS for papers published up to December 2017 using the search terms related to GDM, HbA1c and diagnosis combined. Details of all search terms are presented in **Supplementary Material.** From the papers retrieved, a manual search of their references was conducted. Articles published before 1996, duplicate articles and less complete were removed and the remaining articles were assessed for eligibility. The revision of titles

was followed by the abstracts reading for relevance. Finally, the identification of eligible studies was carried out, based on a full reading of the articles selected by at least 2 researches.

Selection criteria

Inclusion criteria were: (1) cross-sectional or cohort study that assessed the performance of HbA1c (index test) and 75g OGTT (reference test) for the diagnosis of GDM; (2) HbA1c method certified by the National Glycohemoglobin Standardization Program (NGSP; http://www.ngsp.org/, 13 December 2017, date last accessed) and/or International Federation of Clinical Chemistry (IFCC) (14); (3) studies that included pregnant women without DM prior to pregnancy or GDM already diagnosed. Exclusion criteria were: (1) studiesthat did not performe75g OGTT for the diagnosis of GDM; (2) review articles; (3) comments, letters and/or editorials; (4) studies with language other than English, Spanish or Portuguese; (5) articles published before 1996, since it was from this date on that the standardization for the HbA1c methods started (17). Three independent reviewers (P.B.R., F.C.C. and J.R.T.T.) decided for studies inclusion based upon eligibility criteria. First, we screened the titles of all papers resulted from the search to identify potentially relevant articles. Afterwards, we evaluated the abstracts of these studies, and relevant articles had their full-text reviewed. Finally, the reviewers setected articles qualified for inclusion and performed data extraction from all included reports. Any disagreements concerning study eligibility or data interpretation were resolved through discussion or, if required, a fourth reviewer was consulted (J.L.C. or A.L.P.).

Data collection and analysis

A data extraction form was developed and the following information were extracted from each report: (1) study details (author, publication year, country of origin); (2) study design; (3) sample size; (4) GDM incidence; (5) participant characteristics (age, gestational age, HbA1c results); (6) test methods (details of methodology and equipment description for HbA1c and OGTT); and (7) test results [true-positive (TP) cases; false-positive (FP) cases; true-negative (TN) cases; and false-negative (FN) cases]. We also attempted to contact authors for further information when data to construct a 2x2 table was unclear or additional data were required. When data were not available from the authors, the study was excluded.

Quality assessment

At least two reviewers independently assessed the quality of primary studies by evaluating the risk of bias and applicability using the Quality Assessment of Diagnostic Accuracy Studies tool QUADAS-2, a questionnaire containing 14 questions assessing risk of bias and applicability concerns (25). Disagreements were resolved by consensus or by involving a third reviewer (J.L.C. or A.L.P.). We also evaluated if the articles were presented according to Standards for Reporting of Diagnostic

Accuracy (STARD) initiative guidelines (26).

Statistical analysis and data synthesis

We followed the standard methods recommended for diagnostic accuracy meta-analysis studies (27). For each study, 2x2 contingency tables were constructed with data extracted for TP, TN, FP and FN rates. By a bivariate model using a random

effects approach (28) indexes of HbA1c test accuracy were computed: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR). PLR >1 for a positive test result is associated with the presence of disease, and NLR <1 for a negative test result is associated with the absence of disease (29). The DOR is a single indicator that summarizes the diagnostic accuracy of a test, and higher values indicate a better test performance (30). Overall diagnostic accuracy for HbA1c test for GDM diagnosis was determined by a summary receiver operating characteristics curve (SROC) for the main cut-off points discussed in each study. Afterwards, hierarchical summary ROC curves (HSROC) were used to summarize the HbA1c performance for specific cut-offs if 4 or more studies presented data for the same cut-off (31). The Fagan nomogram was applied, considering a pretest probability of 18% for GDM to calculate posttest probabilities for GDM using different HbA1c cut-offs (6, 32). The heterogeneity among studies was evaluated by Chi-square and Cochran Q analysis, I^2 (measure of inconsistency, when I^2 has a value above 50%, it is considered that there is moderate heterogeneity, 25% is low and 75% is high) and by visual inspection of forest plots. When the studies are reasonably homogeneous, the accuracy indexes from individual studies will lie within or near the interval of the pooled accuracy estimate. Deviations may indicate possible heterogeneity or outlier studies (33, 34). We also explored heterogeneity between studies by re-running the meta-analyses removing studies one at a time to determine whether a particular study accounted for the heterogeneity. In addition, when data was available, subgroups analysis by specific cut-off points, HbA1c methods and country of origin were carried out. The presence of publication bias was tested by using Deeks' funnel plots (35). A P-value <0.05 was considered statistically significant in all analyses, except for Deeks' test, where a

value of P <0.1 was considered statistically significant. Analyses were carried out in Meta-Disc Version 1.4 (Universidad Complutense, Madrid, Spain) and Stata Version 12.1 software (Stata, College Station, TX, USA) by METANDI command. The forest plots were constructed using Review Manager Version 5.3 (Cochrane Collaboration, Oxford, UK). All studies selected for this review were previously approved by an Ethical Review Board and consequently ethical approval was not required by this review study.

RESULTS

Study characteristics

The search strategy identified 2,943 records, of those 50 studies were assessed for eligibility. After full-text reading, 41 articles were excluded (one for different language, 9 for insufficient data, 4 for different reference test criterion, 3 studies performed the diagnostic test in the first gestational trimester and 24 didn't meet the research question). Lastly, nine studies met our inclusion criteria, of these, only 1 article (37) was excluded from the meta-analysis due to lacking relevant information to allow a proper extraction of data because it was not clear which diagnostic criterion was used to perform the ROC analysis. Nevertheless, it was included in the qualitative analysis. Eight studies were eligible for systematic review and meta-analysis (20, 22, 38-43) (**Figure 1)**.

All studies included in this review totalized 6,848 pregnant women, who performed OGTT and HbA1c test in the second or third trimesters of pregnancy for GDM diagnosis, of those 1,128 were diagnosed with GDM (15.2%). **Table 1** summarizes the characteristics of all selected studies. Three studies had prospective design (36, 40, 41), one was a retrospective study (42) and five were cross-sectional studies (20, 22, 37-39). All studies were written in English and published between

2005 and 2017. Four studies were from India, and Arab Emirates, Australia, Brazil, China and Turkey contributed with one study each.

Quality Assessment

The quality assessment of the studies by QUADAS-2 criteria is summarized in **Table 2**. Most studies presented a low risk of bias and applicability concerns. One study (37) presented a high risk of bias in the patient selection, flow and timing; in this study 1,459 pregnant women were enrolled, 33 of which were in the first trimester of pregnancy while the remaining women were in the second trimester of pregnancy. Another study (36) had a high risk of bias in the reference standard; this study used two different diagnostic criteria for the diagnosis of GDM and it was not clear which criterion was used in the analyses. For this reason, we did not perform the data extraction. One study (20) presented unclear risk of bias in flow and timing, since 120 pregnant were diagnosed by WHO 1999 diagnostic criteria and 142 were diagnosed by IADPSG criteria. Only one article followed the recommendations and was presented according to the STARD guidelines (20).

Meta-analysis

Overall diagnostic accuracy

For this analysis we considered the main HbA1c cut-offs discussed in each article (20, 22, 37-42). HbA1c threshold ranged from 5.4% to 6.0%. A total of 6,406 pregnant women were included in this analysis, of those 1,044 were diagnosed with GDM. Using data from these eight studies, DOR was 6.97 (95% CI 4.17 – 11.65) and

the area under the curve (AUC) was 0.825 (95% CI 0.751 – 0.899); indicating a good level of overall accuracy (**Figure 2**).

Effect of HbA1c threshold on diagnostic accuracy

The forest plot in **Figure 3** shows the sensitivity and specificity of HbA1c for the detection of GDM across all 8 included studies. For studies reporting accuracy at more than one threshold, 2x2 tables were built for each cutoff. The cutoffs 5.4%, 5.7%, 5.8% and 6.0% were reported by at least 4 studies and their data were included in the forest plots. **Table 3** summarizes the accuracy measures for these cutoffs.

HbA1c ≥5.4% for diagnosis of GDM

Four studies evaluated the cutoff of 5.4% (11, 14, 27, 28), totaling 2,808 pregnant women. HSROC curve showed an AUC of 0.779 (95% CI 0.739 – 0.819; **Figure 4A**). The DOR was 5.20 (95% CI 3.33 – 8.12; I^2 =57.6%). Sensitivity ranged from 26% to 86% and specificity from 61% to 96% (**Figure 3**). The pooled sensitivity for these studies was 50.3% (95% CI 24.8% - 75.7%) and the pooled specificity was 83.7% (95% CI 67.5% to 92.7%) (**Table 3**). After re-running the meta-analysis by removing one paper at a time, when we removed the study by Bhavadharini et al (37), no DOR heterogeneity was found (I^2 = 0%) and pooled sensitivity decreased and pooled specificity was the same [39% (95% CI 33% - 44%) and 83% (95% CI 81% to 84%)], respectively. However, after carefully reviewing this study we were not able to explain the reasons why it contributed to the increase in heterogeneity for this

cutoff and the results from the primary meta-analysis were considered. Considering the prevalence of GDM at 18% (6), for every 1,000 pregnant women tested at a threshold of 5.4%, 91 cases of GDM would be detected, 89 cases will be missed, and there will be 134 false GDM diagnoses.

HbA1c ≥5.7 for diagnosis of GDM

For cutoff of 5.7%, five studies presented data (20, 38, 40, 41), totaling 3,540 pregnant women. HSROC curve showed an AUC of 0.741 (95% CI 0.675 – 0.807; **Figure 4B**). The DOR was 7.03 (95% CI 4.50 – 10.96; I^2 =55.7%). Sensitivity ranged from 9% to 73% and specificity from 76% to 100% (**Figure 3**). The pooled sensitivity for these studies was 24.7% (95% CI 10.3% - 48.5%) and the pooled specificity was 95.5% (95% CI 85.7% to 98.7%) (**Table 3**). After re-running the meta-analysis by removing one paper at a time, no paper explained the moderate DOR heterogeneity for this cutoff and we were unable to explain the reasons for this heterogeneity. Considering the prevalence of GDM at 18% (6), for every 1,000 pregnant women tested at a threshold of 5.7%, 44 cases of GDM would be detected, 136 cases will be missed, and there will be 37 false GDM diagnoses.

HbA1c ≥5.8% for diagnosis of GDM

Four studies evaluated the threshold of 5.8% (37, 38, 20, 42), totaling 4,160 pregnant women. HSROC curve showed an AUC of 0.624 (95% CI 0.482 – 0.766; **Figure 4C**. The DOR was 8.54 (95% CI 4.89 – 14.90; I^2 =38.3%). Sensitivity ranged from 6% to 27% and specificity from 95% to 100% (**Figure 3**). The pooled sensitivity for these studies was 10.8% (95% CI 5.7% - 19.41%) and the pooled specificity was 98.7% (95% CI 96.2% to 99.5%) (**Table 3**). This meta-analysis showed low

heterogeneity thus sensitive analysis was not carried out. Considering the prevalence of GDM at 18% (6), for every 1,000 pregnant women tested at a threshold of 5.8%, 19 cases of GDM will be detected, 161 cases will be missed, and there will be only 11 false GDM diagnoses.

HbA1c ≥6.0% for diagnosis of GDM

Five studies reported data to the threshold of 6.0% (37, 38, 22, 20, 39), totaling 3,608 pregnant women. HSROC curve showed an AUC of 0.927 (95% CI 0.840 – 1.014; **Figure 4D**). The DOR was 11.40 (95% CI 5.34 – 24.36; I^2 =77.0%). Sensitivity ranged from 4% to 47% and specificity from 97% to 100% (**Figure 3**). The pooled sensitivity for these studies was 12.9% (95% CI 5.5% - 27.5%) and the pooled specificity was 98.7% (95% CI 97.6% to 99.3%) (**Table 3**). After re-running the meta-analysis by removing one paper at a time, by removing the study by Saxena et al (39), the DOR heterogeneity was 1.4%. After a careful evaluation, this study was the only one using the WHO 1999 criteria to diagnose GDM instead the IADPSG criteria, this fact could explain the DOR heterogeneity in this subgroup meta-analysis. However, pooled sensitivity and pooled specificity for HbA1c ≥6.0% after excluding this study were practically unchanged and were 10.2% (95% CI 7.6% - 13.2%) and 98.8% (95% CI 98.3% to 99.2%), respectively. Considering the prevalence of GDM at 18% (6), for every 1,000 pregnant women tested at a threshold of 6.0%, 23 cases of GDM will be detected, 157 cases will be missed, and there will be 11 false GDM diagnoses.

Effect of other variables on diagnostic accuracy

We also investigated the effect of different methods of HbA1c measurement and the country of origin of patients to explain the variability among studies. For this analysis, we considered the main HbA1c cut-offs discussed in each article. Four studies used HPLC (27, 11, 31, 32) and 4 used immunoassay (28, 14, 29, 30) to measure HbA1c. We observed low variability when we pooled studies with HbA1c results based only on HPLC methods (DOR =5.48 (95% CI 3.78 – 7.94; I^2 =38.4%). The variability among studies was high when we pooled only immunoassay methods (DOR =8.38 (95% CI 2.79 – 25.1; I^2 =88.6%), however when we excluded the study by Saxena et al (39) a low heterogeneity was observed (DOR =4.92 (95% CI 3.12 – 7.75; I^2 =11.3%). The heterogeneity was also low when we pooled only studies from Asia (37, 22, 39-42) [DOR =4.77 (95% CI 3.55 – 6.40; I^2 =38.4%) and absent when we evaluated non-Asian studies (20, 39) [DOR =7.21 (95% CI 4.15 – 12.54; I^2 =0.0%)].

Publication Bias

Although investigation of reporting bias in diagnostic accuracy data is not well established, we used the method of Deeks (35), that appears to be more appropriate, which indicated that there was no potential publication bias (p =0.112).

Probabilities post-test

Considering the pre-test probability of 18% for GDM and the PLR and NLR for cutoffs 5.4%, 5.7%, 5.8% and 6.0% we calculated the post-test probabilities for GDM applying the Fagan´s normogram (**Figure 5**). The post-test probabilities were 40% and 12% for HbA1c ≥5.4% and <5.4%; 55% and 15% for HbA1c ≥5.7% and <5.7%;

64% and 17% for HbA1c ≥5.8% and <5.8%; 69% and 16% for HbA1c ≥6.0% and <6.0%; respectively.

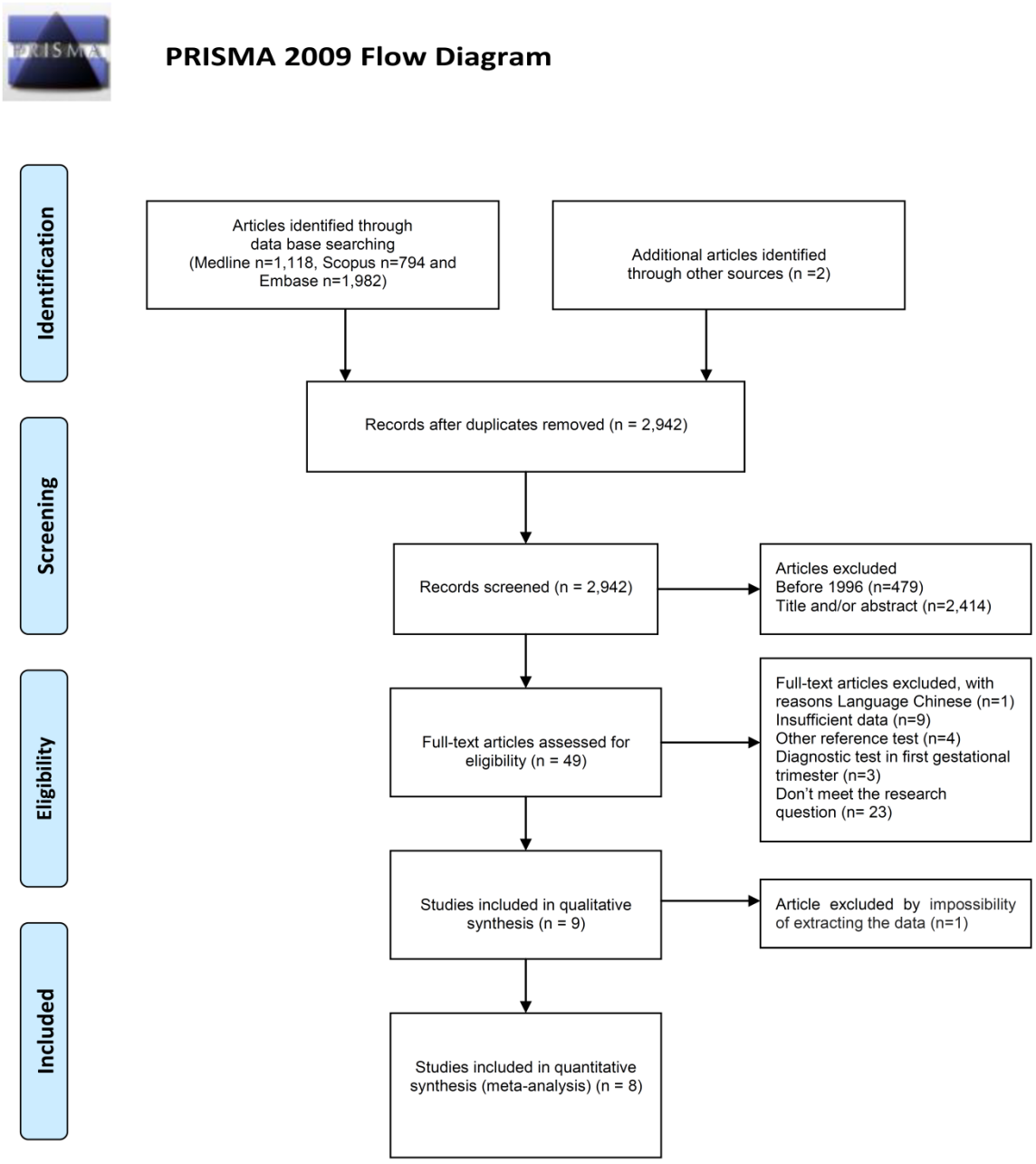

Figure 1: Flowchart of the article selection process.

Table 1. Characteristics of selected studies.

Reference	Country	Study design	Age (years)	Diagnostic criteria 75g OGTT	HbA1c (%)	GDM incidence (%)	Pregnancy weeks	HbA1c cut-off (%)***	Total N	HbA1c method
Agarwal 2005	Árab Emirates	Prospective	26.6(±5.5)	WHO/ADA	Without GDM 5.95(±0.75) With GDM 6,0 (±0.81)	19 by WHO 11 by ADA	24-28	6.0/5.0/ **5.5**	442	Immunoassay LX20 SynchronPro
Bhavadharini 2017*	Índia	Cross-sectional	26.1(±3.9)	IADPSG	Without GDM 4.9(±0.5) With GDM 5.2(±0.5)	13	12-26	5.0/**5.8**/6.0	1459	HPLC (Variant II Turbo analyzer) Biorad Laboratories
Khalafallah 2016	Australia	Cross-sectional	18-47	ADIPS	4,8 (±0.36)	12	24-28	**5.4**/5.1/ 5.8	480	Imunoassay by DCA 2000 Siemens
Rajput 2012	Índia	Cross-sectional	16-30	ADA/IADPSG	With GDM 5.73(±0.34)	7 byADA 24 byIADPSG	24-28	**5.95**/5.45	607	Immunoassay Conelab 30 i
Renz 2015**	Brazil	Cross-sectional	23-35	WHO and/or IADPSG	Without GDM 5.1(±0.4) With GDM 5.5(±0.5)	33	22-32	5.3/**5.8**	262	HPLC (Variant II Turbo analyzer) Biorad Laboratories
Saxena 2017	Índia	Cross-sectional	25(±3.6)	WHO/DIPSI	Without GDM 5.06(±0.54) With GDM 6.43(±0.78)	6.38	24-32	**6.0**	800	Immunoassay AU480, Randox reagent
Servket 2014	Turkey	Prospective	27.9(±5.2)	IADPSG	Without GDM 5.0(±0.5) With GDM 5.5(±0.7)	16	24-28	5.2/**5.7**	339	Immunoassay Roche Hitachi Tokio
Soumya 2015	Índia	Prospective	NoGDM 25.8(±3.1) GDM 28.6 (±1.2)	IADPSG	Without GDM 5.4(±0.5) With GDM 6.2(±0.6)	9	24-28	5.3/**5.7**	500	HPLC BioRad Laboratories, Hercules CA,USA
Ye 2016	China	Retrospective	No GDM 29.5(±3.7) GDM 31.6 (±4.3)	IADPSG	Without GDM 4.9(±0.3) With GDM 5.1(±0.4)	21	24-28	5.3/**5.5**	1959	HPLC (Variant II Turbo analyzer) Biorad Laboratories

Data are expressed as mean±SD or range. *33 pregnant in 1stgestational trimester; **120 pregnant by WHO 1999 diagnostic criteria; *** principal HbA1c cut-off in bold; OGTT (Oral glucose tolerance test); ADA (American Diabetes Association); WHO (World Health Organization); IADPSG (International American Diabetes Pregnancy Study Group); DIPSI (Diabetes in Pregnancy Study group India); ADIPS (Australasian Diabetes in Pregnancy Society).

Table 2. Quality assessment using QUADAS-2 criteria.

Checklist QUADAS-2

Study	Risk of Bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
1.Agarwal 2005	☺	☺	☹	?	☺	☺	☺
2.Bhavadharini 2017	☹	☺	☺	☹	☺	☺	☺
3.Khalafallah 2016	☺	☺	☺	☺	☺	☺	☺
4.Rajput 2012	☺	☺	☺	☺	☺	☺	☺
5.Renz 2015	☺	☺	☺	?	☺	☺	☺
6.Saxena 2017	☺	☺	☺	☺	☺	☺	☺
7.Servket 2014	☺	☺	☺	☺	☺	☺	☺
8.Soumya 2015	☺	☺	☺	☺	☺	☺	☺
9.Ye 2016	☺	☺	☺	☺	☺	☺	☺

☺ low risk; ☹ high risk; ? unclear risk.

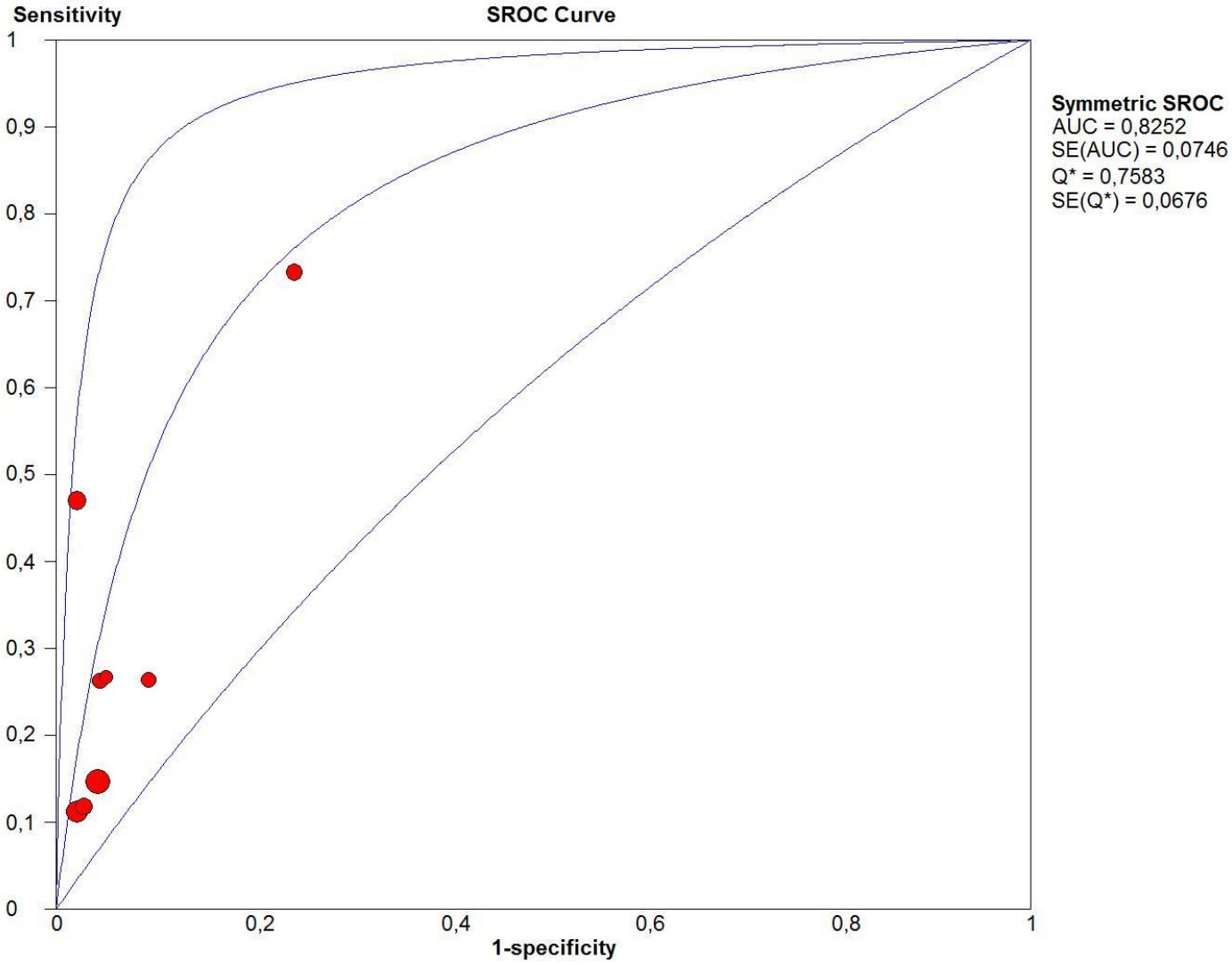

Figure 2. Summary receiver operating characteristic curves (SROC) of HbA1c for all 8 studies.

Table 3. Pooled diagnostic accuracy measures of different HbA1c cut-offs for GDM

	HbA1c cut-off (%)			
Pooled indexes	**5.4**	**5.7**	**5.8**	**6.0**
Sensitivity	0.503 (0.25- 0.76)	0.247 (0.10- 0.48)	0.107 (0.06- 0.19)	0.129 (0.05- 0.27)
Specificity	0.837 (0.67 - 0.93)	0.955 (0.86 - 0.99)	0.987 (0.96 - 0.99)	0.987 (0.98 - 0.99)
PLR	3.09	5.5	8.09	10.06
NLR	0.59	0.79	0.90	0.88
DOR	5.20 (3.33 - 8.12)	7.03 (4.5 - 10.96)	8.95 (5.17 - 15.50)	11.40 (5.34 - 24.36)
AUC	0.779	0.741	0.626	0.927

PLR= positive likehood ratio; NLR=negative likehood ratio; DOR= diagnostic odds ratio; AUC= area under the curve.

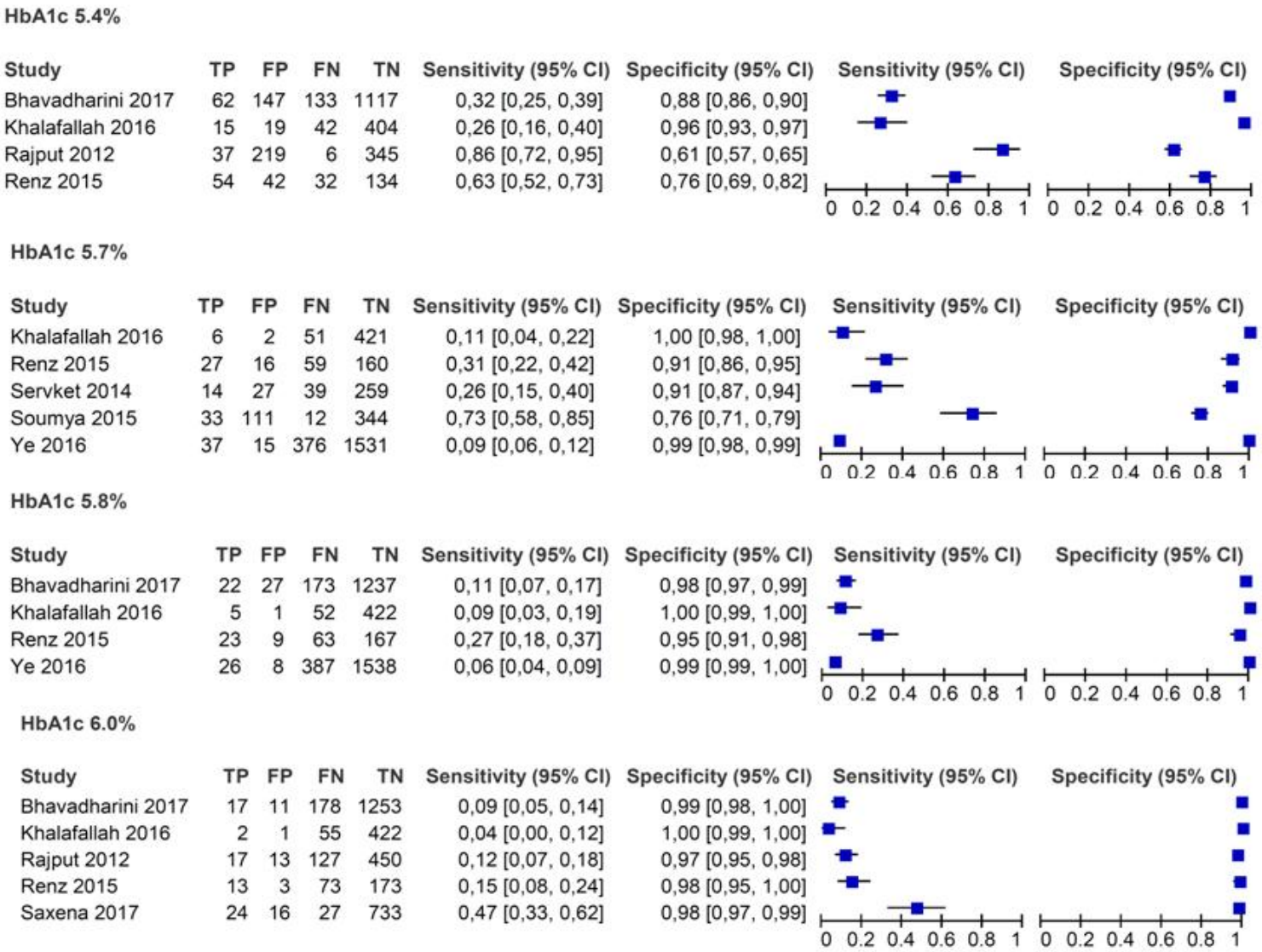

Figure 3 Forest plot of the sensitivity and specificity of HbA1c cut-offs in the diagnosis of GDM. TP = true positive; FP = false positive; FN = false negative; TN = true negative. The blue square depicts the sensitivity and specificity for each study and the horizontal line represents the corresponding 95% confidence interval for these estimates

Table 4. Summary of findings

Review question: What is the diagnostic accuracy of HbA1c in the GDM diagnosis?				
Population: Pregnant women in prenatal care, without previous DM, in the third trimester of pregnancy				
Studies: cross-sectional diagnostic test accuracy studies, cohort studies, reporting 2x2 data				
Index test: HbA1c				
Reference standard: 75 g oral glucose tolerance test - OGTT				
Subgroup **HbA1c (%)**	**Studies (N)**	**Sensitivity (95%IC)**	**Specificity (95%IC)**	**Interpretation for every 1,000 pregnant women tested** **Prevalence = 18%**
5.4	4	0.503 (0.25- 0.76)	0.837 (0.67 - 0.93)	91 cases of GDM will detected, 89 cases will be missed, and there will be 134 false GDM diagnoses
5.7	5	0.247 (0.10- 0.48)	0.955 (0.86 - 0.99)	44 cases of GDM will detected, 136 cases will be missed, and there will be 37 false GDM diagnoses
5.8	4	0.107 (0.06- 0.19)	0.987 (0.96 - 0.99)	19 cases of GDM will detected, 161 cases will be missed, and there will be only 11 false GDM diagnoses
6.0	5	0.129 (0.05- 0.27)	0.987 (0.98 - 0.99)	23 cases of GDM will detected, 157 cases will be missed, and there will be 11 false GDM diagnoses

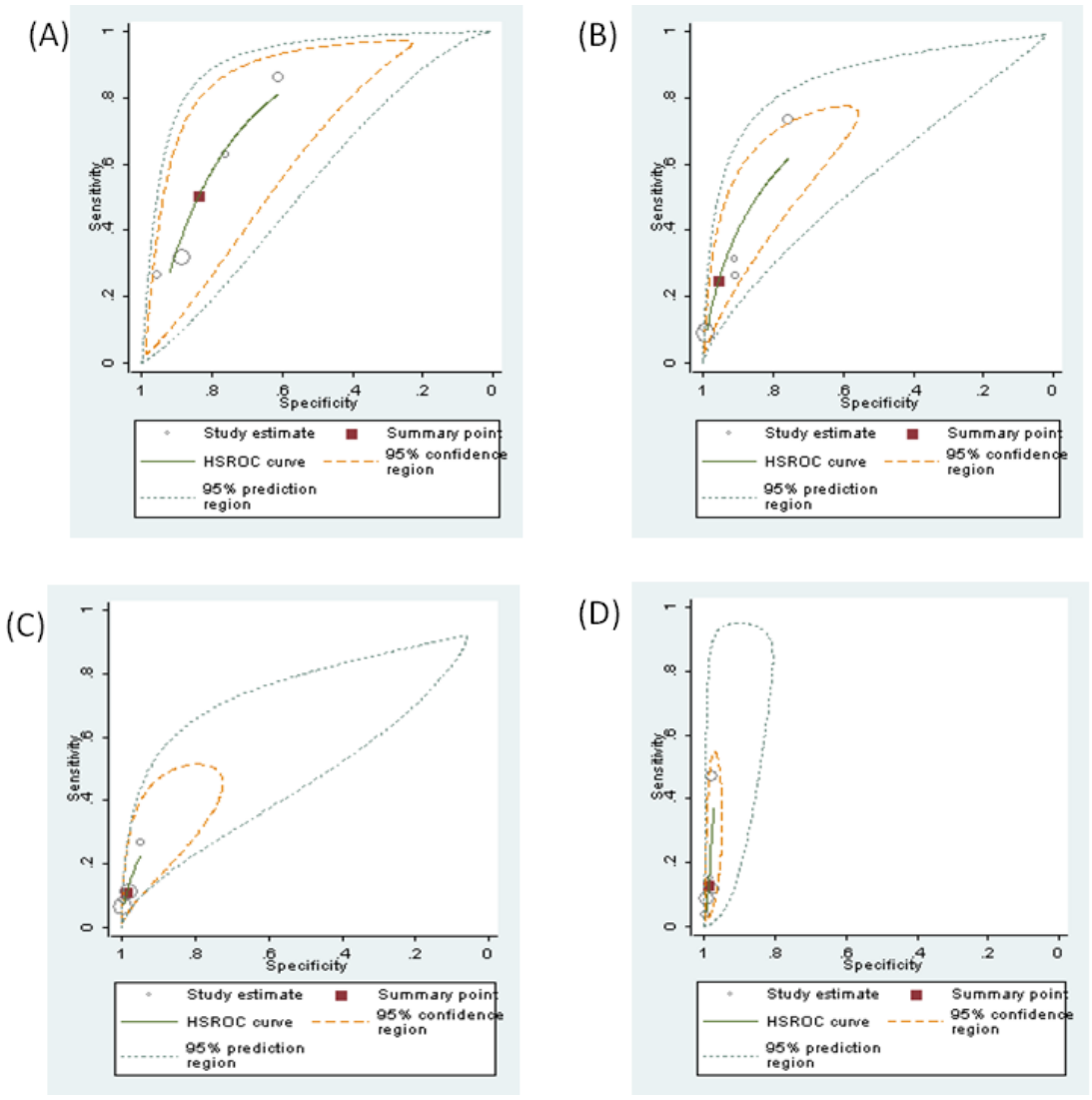

Figure 4. Hierarchical summary receiver operating characteristic curves of HbA1c at different cut-off points for GDM. A) 5.4% B) 5.7% C) 5.8% D) 6.0%.

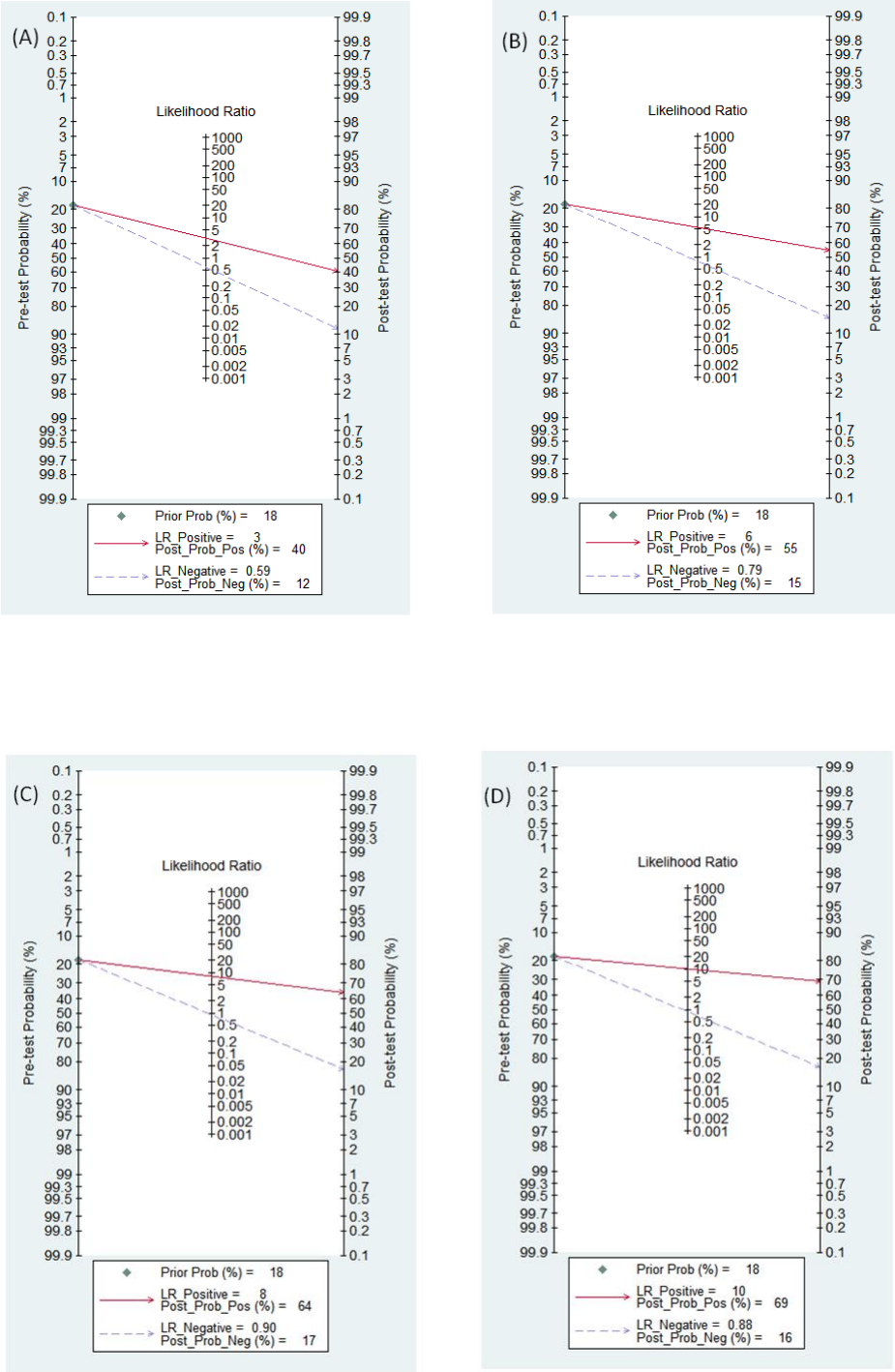

Figure 5. Fagan's normograms for HbA1c test, showing posttest probabilities for GDM. (A) HbA1c ≥5.4%, (B) HbA1c ≥5.7%, (C) HbA1c ≥5.8% and (D) HbA1c ≥6.0%.

DISCUSSION

Summary of main results

In this meta-analysis we include 8 studies, covering 6,406 pregnant women, of those 1,044 were diagnosed with GDM. The diagnostic accuracy of HbA1c was reported at different thresholds ranging from 5.4% to 6.0%. AUC was 0.825 (95% CI 0.751 – 0.899) with a Q* value of 0.758, indicating a good level of overall accuracy of HbA1c test. Four studies evaluated the cutoff of 5.4% (20, 22, 37, 38), totaling 2,808 pregnant women. The pooled sensitivity and specificity for these studies was 50.3% (95% CI 24.8% - 75.7%) and 83.7% (95% CI 67.5% to 92.7%), respectively. For cutoff of 5.7%, five studies presented data (20, 38, 40, 41), totaling 3,540 pregnant women. Pooled sensitivity and specificity for these studies was 24.7% (95% CI 10.3% - 48.5%) and 95.5% (95% CI 85.7% to 98.7%), respectively. Four studies evaluated the threshold of 5.8% (38, 39, 20, 43), totaling 4,160 pregnant women yielding a pooled sensitivity and specificity of 10.8% (95% CI 5.7% - 19.41%) and 98.7% (95% CI 96.2% to 99.5%). Five studies reported data for the threshold of 6.0% (38, 39, 22, 20, 40), totaling 3,608 pregnant women. The pooled sensitivity and specificity for these studies was 12.9% (95% CI 5.5% - 27.5%) and 98.7% (95% CI 97.6% to 99.3%), respectively. We also have tabulated a summary of the main results from this review in **Table 4**.

Our results compared with other reports

As far as we know this is the first meta-analysis including a multi-ethnic population to evaluate the accuracy of HbA1c test in the diagnosis of GDM. A recent

study in Chinese pregnant women (18) that aimed to establish the overall accuracy of HbA1c for the diagnosis of patients with GDM, after systematic review, included 5,918 controls and 2,812 patients with GDM. Meta-analyzed data in this report showed sensitivity of 0.762 (95% CI 0.746 – 0.777), specificity of 0.917 (95% CI 0.910 - 0.924) and an AUC of 0.93 with a Q*value of 0.841, indicating also a high level of overall accuracy for HbA1c test in the diagnosis of GDM.

In a prospective study that enrolled 1,989 pregnant Taiwanese women (43), the AUC was 0.70 and the optimal HbA1c cut-off point to predict GDM was 5.7% (sensitivity = 45.2% and specificity = 84.1%). However, the reference test adopted in this study was the two steps OGTT recommended by National Health Institute (NHI), though these results are in agreement with this review, showing low sensitivity and relative high specificity for HbA1c to diagnose GDM. Additionally, the study by Li et al (44) reported a positive correlation of HbA1c with blood glucose in pregnancy affected by GDM. They showed an AUC for HbA1c of 0.854 ($P<0.01$). When HbA1c was 5.43%, sensitivity and specificity were 0.832 and 0.764, respectively. Hanna et al (46) examined the concordance between different criteria for GDM diagnosis and observed an increased proportion of women with an HbA1c ≥6.0% in the discordant cases. They, then, evaluated the performance of this HbA1c threshold in the diagnosis of GDM and found a similar sensitivity and specificity of HbA1c, around 22% and 97%, respectively, irrespective of the criteria used to diagnose GDM. They concluded that HbA1c test alone is unlikely to replace the OGTT in GDM diagnosis. Indeed, an optimal test to diagnose GDM is still desired. The recent study by Farrar et al (46) evaluated by a systematic review different test strategies for the diagnosis of GDM and concluded that there is insufficient evidence to suggest which strategy is best for diagnosing GDM, although HbA1c data were not included in this study.

Strengths and weaknesses of the review

This study was conducted through an extensive and systematic literature search, we included papers from different countries that analyzed different populations of pregnant women. At least two independent reviewers extracted the data and the overall quality of original studies was checked by a QUADAS-2 tool to perform quality assessments and most studies presented low risk of bias and applicability concerns. As limitations for this study, we highlight: First, although we only included studies that measured HbA1c with standardized methods, the individual performance of each laboratory was not available. Second, we observed a high heterogeneity among the studies, mainly regarding data for HbA1c sensitivity. We should draw attention to the likely effect of ethnicity and the use of different criteria for OGTT interpretation. One study used the WHO 1999 criteria (29) and after its exclusion a low heterogeneity was observed. The heterogeneity was also low when we pooled only studies from Asia (22,37,39,40,41,42) and absent when we evaluated non-Asian studies (20,38). Third, only one article (20) followed the recommendations and was presented according to the STARD guidelines (26) which may be affected the quality of reporting of the other studies.

Applicability of findings to the review question

To make sense for the results of the meta-analysis and to calculate the false-error rates, we calculated the post-test probabilities for GDM applying the Fagan´s normogram, we considered the test performance estimates based on external data from the Metzger et al study (6), with pre-test probability of 18% for GDM and the PLR and NLR for cutoffs 5.4%, 5.7%, 5.8% and 6.0%. The post-test probabilities for

a positive test were 40%, 55%, 64% and 69% for HbA1c ≥5.4%, HbA1c ≥5.7%, HbA1c ≥5.8% and HbA1c ≥6.0%; respectively. The post-test probabilities for a negative test for these cutoffs were low and ranged from 12 to 17%, similar to the pre-test probability of 18%. HbA1c results ≥5.4% increase at least 2-fold the probabilitiy for GDM whereas HbA1c results <5.4% do not alter the initial probability of GDM.

Conclusion

Limited evidence provided by the studies included in this review suggests that HbA1c tests, regardless of the threshold used to diagnose GDM, result in few false-positives GDM cases but very high levels of false negativeGDM cases, with a high level of specificity across all population groups described here. These findings point out to the usefulness of HbA1c as a rule-in test for the diagnosis of GDM. However it means that a negative result will require further investigation through a more sensitive test for confirmation of diagnosis. The prognostic value of HbA1c for GDM adverses outcomes still has to be evaluated by prospective studies.

Supplementary Material

Pubmed:((((((((((((((laboratory test) OR laboratory diagnosis) OR Tests, Diagnostic) OR Test, Diagnostic) OR Diagnostic Test) OR Diagnostic Tests) OR Routine Diagnostic Test) OR Diagnostic Test, Routine) OR Test, Routine Diagnostic) OR Routine Diagnostic Tests) OR Tests, Routine Diagnostic) OR Diagnostic Tests, Routine) OR Diagnosis)) AND ((((((((((((((((((hemoglobin a (1c)) OR hba1c) OR hba 1c) OR hb a (1c)) OR haemoglobin A1c) OR haemoglobin a (1c)) OR haemoglobin a 1c) OR glycosylated hemoglobin A1c) OR glycosylated haemoglobin A1c) OR glycated hemoglobin A1c) OR glycated haemoglobin A1c) OR Hemoglobin A1c) OR Hemoglobins, Glycated) OR Glycated Hemoglobins) OR Glycosylated Hemoglobin) OR Hemoglobin, Glycosylated) OR Glycohemoglobin A) OR Glycosylated Hemoglobin A) OR Hb A1c) OR Hemoglobin A, Glycosylated)) AND ((((((((((((pregnancy diabetes) OR diabetes, pregnancy) OR diabetes mellitus gravidarum) OR maternal diabetes mellitus) OR pregnancy diabetes mellitus) OR Gestational Diabetes Mellitus) OR Diabetes Mellitus, Gestational) OR Gestational Diabetes) OR Pregnancy-Induced Diabetes) OR Diabetes, Pregnancy Induced) OR Diabetes, Pregnancy-Induced) OR Diabetes, gestational) OR Gestational diabetes)

EMBASE: gestational AND ('diabetes'/exp OR diabetes) OR diabetes, AND gestational OR diabetes, AND 'pregnancy induced' OR diabetes, AND ('pregnancy'/exp OR pregnancy) AND induced OR 'pregnancy induced' AND ('diabetes'/exp OR diabetes) OR gestational AND ('diabetes'/exp OR diabetes) OR 'diabetes'/exp OR diabetes AND mellitus, AND gestational OR gestational AND ('diabetes'/exp OR diabetes) AND mellitus OR 'pregnancy'/exp OR pregnancy AND ('diabetes'/exp OR diabetes) AND mellitus OR maternal AND ('diabetes'/exp OR diabetes) AND mellitus OR 'diabetes'/exp OR diabetes AND mellitus AND gravidarum OR diabetes, AND ('pregnancy'/exp OR pregnancy) OR 'pregnancy'/exp OR pregnancy AND ('diabetes'/exp OR diabetes) AND 'hemoglobin'/exp OR hemoglobin AND a, AND glycosylated OR 'hb'/exp OR hb AND a1c OR glycosylated AND ('hemoglobin'/exp OR hemoglobin) AND a OR 'glycohemoglobin'/exp OR glycohemoglobin AND a OR hemoglobin, AND glycosylated OR glycosylated AND ('hemoglobin'/exp OR hemoglobin) OR glycated AND ('hemoglobins'/exp OR hemoglobins) OR hemoglobins, AND glycated OR 'hemoglobin'/exp OR hemoglobin AND a1c OR glycated AND ('haemoglobin'/exp OR haemoglobin) AND a1c OR glycated AND ('hemoglobin'/exp OR hemoglobin) AND a1c OR glycosylated AND ('haemoglobin'/exp OR haemoglobin) AND a1c OR glycosylated AND ('hemoglobin'/exp OR hemoglobin) AND a1c OR 'haemoglobin'/exp OR haemoglobin AND a AND 1c OR 'haemoglobin'/exp OR haemoglobin AND a AND 1c OR 'haemoglobin'/exp OR haemoglobin AND a1c OR 'hb'/exp OR hb AND a AND 1c OR hba AND 1c OR 'hba1c'/exp OR hba1c OR 'hemoglobin'/exp OR hemoglobin AND a AND 1c AND 'diagnosis'/exp OR diagnosis OR 'diagnostic'/exp OR diagnostic AND tests, AND routine OR tests, AND routine AND ('diagnostic'/exp OR diagnostic) OR routine AND ('diagnostic'/exp OR diagnostic) AND tests OR test, AND routine AND ('diagnostic'/exp OR diagnostic) OR 'diagnostic'/exp OR diagnostic AND test, AND routine OR routine AND ('diagnostic'/exp OR diagnostic) AND ('test'/exp OR test) OR 'diagnostic test'/exp OR 'diagnostic test' OR 'diagnostic'/exp OR diagnostic AND ('test'/exp OR test) OR test, AND ('diagnostic'/exp OR diagnostic) OR tests, AND ('diagnostic'/exp OR diagnostic) OR 'laboratory'/exp OR laboratory AND ('diagnosis'/exp OR diagnosis) OR 'laboratory'/exp OR laboratory AND ('test'/exp OR test)

SCOPUS: (((TITLE-ABS-KEY (diagnosis)) OR (TITLE-ABS-KEY (diagnostic AND tests, AND routine)) OR (TITLE-ABS-KEY (tests, AND routine AND diagnostic)) OR (TITLE-ABS-KEY (routine AND diagnostic AND tests)) OR (TITLE-ABS-KEY (test, AND routine AND diagnostic)) OR (TITLE-ABS-KEY (diagnostic AND test, AND routine))) OR ((TITLE-ABS-KEY (routine AND diagnostic AND test)) OR (TITLE-ABS-KEY (diagnostic AND tests)) OR (TITLE-ABS-KEY (diagnostic AND test)) OR (TITLE-ABS-KEY (test, AND diagnostic)) OR (TITLE-ABS-KEY (tests, AND diagnostic)) OR (TITLE-ABS-KEY (laboratory AND diagnosis)) OR (TITLE-ABS-

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7. ARTIGO III

Glycated hemoglobin (HbA1c) in pregnancy and adverse maternal-fetal outcomes

Glycated hemoglobin (HbA1c) in pregnancy and adverse maternal-fetal outcomes

Paula Breitenbach Renz [1]

MayanaKieling Hernandez [1]

Angela Jacob Reichelt [1,2]

Joíza Lins Camargo [1,2*]

[1] GraduateProgram in Endocrinology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

[2] EndocrinologyDepartment, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil

* Corresponding Author:

E-mail address: jcamargo@hcpa.edu.br

Abstract

Background: Gestational diabetes mellitus (GDM) when uncontrolled is associated with spontaneous abortion, fetal anomalies, preeclampsia, fetal death, macrosomia, neonatal hypoglycemia, and neonatal hyperbilirubinemia, among others. Our objective is to analyze the relationship between the levels of HbA1c and adverse events during pregnancy and in the delivery.

Methods: 299 pregnant women in prenatal care, without prior DM, who underwent OGTT test with 75g glucose overload in the third trimester of pregnancy, were invited to participate and those who had the delivery performed in our institution had outcomes evaluated. HbA1c was determined by an HPLC method and 75 g OGTT was carried out in accordance with the recommendations of WHO2013. The maternal and neonatal adverse outcomes, such as cesarean delivery, maternal hypertension, preterm delivery, macrosomia, neonatal morbidity and intensive care hospitalization were analyzed by quartiles of HbA1c.

Results: A total of 276 pregnant women participated in the study, of those 75 were diagnosed with GDM by OGTT. Eighty-nine pregnant women in first quartile presented HbA1c values ≤4.9%, 108 (second and third quartile) had HbA1c values between 5.0 and 5.3% and 79 (fourth quartile) had HbA1c ≥5.4%. Women presenting HbA1c ≥5.4% had higher incidences of maternal hypertension (39% vs 18%, P=0.036) and GDM (53.2% vs 10.1%, P=0.000) in comparison with those with HbA1c ≤4.9%. After adjustment, relative risk (RR) for maternal hypertension was 1.44 (0.853 – 2.442) and 1.56 (0.966 – 2.516) in the groups with HbA1c ≥5.4% and HbA1c 5.0-5.3%, respectively.

Conclusions: HbA1c levels are associated with hypertensive outcomes of pregnancy. However this association is not significant when adjusted for pregnancy BMI (Kg/m^2), maternal age, hypertension prior to pregnancy and GDM. Our results did not show association of HbA1c levels and other materno-fetal averse outcomes, though we also observed an increase in the frequency of adverse neonatal events as macrosomia and neonatal morbidity, in those women in the higher quartiles of HbA1c.

Key words: Gestational diabetes *mellitus*, HbA1c, adverse pregnancy outcomes.

Introduction

Gestational diabetes mellitus (GDM) is defined as the diabetes diagnosed in the second or third trimester of pregnancy, excluding the possibility of type 1 diabetes (DM1) or type 2 (DM2) (1). Among the risks related to the presence of uncontrolled diabetes in pregnancy are spontaneous abortion, fetal anomalies, preeclampsia, fetal death, macrosomia, neonatal hypoglycemia, neonatal hyperbilirubinemia, among others (2). From the obstetric point of view, there are outcomes such as hypertensive disease, polyhydramnios, premature labor, hypoglycemia, shoulder dystocia, fetal death and an increase in the number of cesarean sections due to macrosomia (baby weight greater than 4 kg). Regardless of the presence of risk factors, the World Health Organization (WHO) recommends the screening of GDM for all pregnant women (3). In 2010, the International Association of Diabetes and Pregnancy Study Group (IADPSG) recommended that the diagnostic criteria for GDM be based on the results of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study (4). Thus, the following cutoff points were suggested for the diagnosis of GDM in the oral glucose tolerance test (OGTT): fasting, 1h and 2h of 92 mg/dL, 180 mg/dL and 153 mg/dL, respectively, at least one point altered is diagnostic for GDM. These criteria were adopted in the ADA 2011 recommendations and endorsed by WHO (5, 6).

The use of the HbA1c has some advantages such as the test allows the collection of a single blood sample; fasting is not required and is very practical. It seems that it can predict diabetes complications practicality as well as glycemia tests. Hughes et al showed in a cohort study that HbA1c score ≥5.9% in pregnant women with GDM with a mean of 47 days of gestation was a predictor of risk for congenital anomalies, preeclampsia, shoulder dystocia and perinatal death (7). Sweeting et al. also observed that pregnant women with GDM and HbA1c >5.9% were at an

increased risk of adverse events in pregnancy (large babies for gestational age (LGA), macrosomia, cesarean section and hypertensive disorders) in addition to neonatal hypoglycemia rates when compared to the other categories of HbA1c (8). Results from the HAPO study showed that there is a continued risk of adverse outcomes and maternal blood glucose levels, with no specific glycaemia point related to each outcome (9). Similar to HAPO study, the Sweting study demonstrated an association between higher levels of HbA1c and outcomes, although OGTT was used as the standard diagnosis (8). We studied the role of HbA1c as diagnostic test for GDM, and our results showed that 38% of the cases of GDM were diagnosed using the cutoff point of HbA1c ≥5.8%, this cut-off point had sufficient specificity to confirm the diagnosis (10).

In this study we aimed to analyze the relation between HbA1c levels in the third trimester of gestation and adverse maternal-fetal events during pregnancy and delivery.

Patients and Methods

Patients

This is a longitudinal study to evaluate the prevalence of GDM adverse outcomes according to HbA1c levels. It was conducted from October 2009 to November 2017 in the Division of Endocrinology of the Hospital de Clinicas de Porto Alegre (HCPA) and consisted of two stages:

Stage 1 - Pregnant women in prenatal care, without prior DM, who underwent OGTT test with 75g glucose in the third trimester of pregnancy, were invited to participate. All women signed an informed consent term and answered a standardized

questionnaire. The study protocol was approved by the Ethics Committee of the Hospital de Clinicas de Porto Alegre (GPPG-HCPA), under protocol number 14-0579. Patients younger than 18 years of age were excluded from this study. We also excluded patients with clinical conditions known to interfere or lead to misinterpretation of HbA1c results, such as chronic kidney disease, moderate (Hb ≥7 g/dL and <10 g/dL) and severe anemia (Hb <7 g/dL), or with presence of hemoglobin variants (11, 12). Pregnant women with twin pregnancies were also excluded. After an eight-hour fast, blood samples were collected to determine HbA1c levels, blood cell counts, and glucose concentrations. Blood pressure was checked at the beginning of the interview through an automatic blood pressure monitor with arm cuff (HEM742INT, Omrom, MS80047300098).

Stage 2 – Clinical evaluations of pregnant, delivery and newborns were used to identify maternal-fetal adverse outcomes. After delivery, the medical records were reviewed to observe the following adverse outcomes:

- maternal: cesarean section, gestational hypertension, preeclampsia, eclampsia and death. The hypertensive outcomes of pregnancy**,** was defined as the presence of gestational hypertension, preeclampsia or eclampsia.
- neonatal: preterm birth, birth weight (large or small for gestational age), macrosomia (baby weight ≥4 kg), need for any neonatal care, hypoglycemia, respiratory complications and death. The neonatal morbidity outcome was defined as the presence of birth weight (large or small for gestational age), macrosomia, need for any neonatal care or hypoglycemia).

Laboratory Analysis

Hemogram was performed by flow cytometry (ABX Pentra DX 120, HORIBA, Kyoto, Japan).The 75g OGTT was carried out in accordance with the recommendations of WHO 2013 (6). Before 2011 June, the GDM diagnosis at our institution follows the old protocol by recommendations of the 2nd Meeting of the Diabetes and Pregnancy Task Force (12) using the cut-off points of FPG ≥110mg/dL (6.1 mmol/L) or G2h ≥140mg/dL (7.8 mmol/L). After 2011 July, GDM was diagnosed by ADA/IADPSG criteria (one in three of the following cutoff points: FPG ≥92mg/dL (5.1 mmol/L), 1h glycemia ≥180mg/dL (10.0 mmol/L) and 2h glycemia ≥153mg/dL (8.5 mmol/L). Anemia was classified by WHO 2011 criteria. HbA1c was determined by an HPLC method (Variant II Turbo HbA1c, BioRad Laboratories, Hercules, CA, USA). This method is certified by the National Glycohaemoglobin Standardization Program (NGSP) and is aligned with the reference method of the International Federation of Clinical Chemistry (IFCC) (http://www.ngsp.org/ifcc.asp).

Statistical Analysis

Data were expressed as mean and SD for normally distributed variables, and as median (range) for non-Gaussian variables. The incidence of the outcomes were calculated. T test, Kruskall Wallis test, chi square test were used when appropriate. Multiple Poisson regressions were performed, with maternal/fetal outcomes as dependent variables, in separate models, and HbA1c as independent variables. All data were analyzed with IBM SPSS software for Windows, version 19.0 (Statistical Package for Social Sciences - Professional Statistics, IBM Corp., Armonk, USA).

Sample Size Estimation: Based on the study by O'Sullivan EP et al, which evaluated the impact of the new diagnostic criteria recommended by IADPSG on maternal-fetal outcomes in European pregnant women (13), and in the study already conducted by our group, which evaluated HbA1c test in the diagnosis of GDM (10), the number of pregnant women required for this longitudinal study was calculated. The total N was determined according to the outcomes weeks of gestation and birth weight, with a sample estimated of 200 and 155 pregnant women, respectively, with a power of 80% and a significance level of 5%.

Results

A total of 299 pregnant women were recruited for this study, 23 were excluded – 5 had DM, 10 presented moderate to severe anemia, and 8 had birth delivery carried outside our Institution (**Figure 1**). Of the 276 pregnant women who participated in the study, 188 were evaluated by ADA/IADPSG criteria (recruited after July 2011) and 88 were evaluated by our institutional protocol (13). Seventy and five women were diagnosed with GDM, of those, 42 were diagnosed by ADA/IADPSG criteria and 33 by local recommendations. Baseline characteristics of all participant women are depicted in **Table1**.

Table 2 shows maternal and neonatal adverse outcomes according to the quartiles of HbA1c, where interval of HbA1c 5.0-5.3% represents second and third quartile together. Eighty-nine pregnant women presented HbA1c values ≤4.9%, 108 had HbA1c values between 5.0 and 5.3% and 79 had HbA1c ≥5.4%. Cesarean delivery was observed in 55% of the pregnant women with HbA1c values ≤4.9%, in 50% of the pregnant women with HbA1c between 5.0 and 5.3% and in 60% of the pregnant women with ≥5.4% HbA1c (P =0.095). In relation to the presence of

hypertensive outcomes of pregnancy, we observed the occurrence of these events in 18% of pregnant women with HbA1c ≤4.9%, in 30% of women with HbA1c between 5.0 and 5.3% and in 39% of women with HbA1c ≥5,4% (P =0.036). Sixteen women were already hypertensive before pregnancy, 6 in the group with HbA1c ≤4.9%, 6 in the group with HbA1c between 5.0 and 5.3% and 4 in the group with HbA1c ≥5.4%.

Of those pregnant women with GDM, 73 were under DM treatment at the time of delivery (38 only diet, 27 oral hypoglycemic agents, 3 insulin and 5 insulin associated with oral agents). As expected, the use of GDM therapy increases with HbA1c levels (P <0.001). GDM diagnosis was observed in 10.1% of women with HbA1c levels ≤4.9%, 22.2% of women with HbA1c levels between 5.0 and 5.3%, and 53.2% of pregnant women with HbA1c≥5.4% (P <0.001).

We observed the occurrence of preterm birth in 15.7%, 20.4% and 25.3% in the groups with HbA1c ≤4.9%, HbA1c from 5.0 to 5.3% and ≥5.4%, respectively (P =0.393). Macrosomia occurred in 4.5%, 6.5% and 6.3% of pregnant women with HbA1c ≤4.9%, HbA1c 5.0 -5.3% and HbAc1 ≥5.4%, respectively (P =0.815). The frequency of neonatal morbidity was 22.5%, 21.3% and 32.9% in the groups with HbA1c ≤4.9%, HbA1c 5.0 -5.3% and HbA1c ≥5.4%, respectively (P =0.155). The intensive care unit (ICU) hospitalization was observed in 13.5%, 13.9% and 16.5% of groups with HbA1c ≤4.9%, HbA1c 5.0 - 5.3% and hbA1c ≥5.4%, respectively (P =0.839). Five infants presented malformation (2 esophageal atresia, 2 gastroschisis and 1 ventriculomegaly), 2 of them were born from mothers with GDM.

Multiple Poison regressions were conducted to examine the association between the HbA1c levels and risk of adverse pregnancy outcomes. The results are shown in **Table 3**. In the unadjusted model analyses there was significant association of maternal hypertension with high levels of HbA1c (HbA1c ≥5.4 and HbA1c 5.0–5.3)

compared to HbA1c ≤4.9%, Women in the higher quartile of HbA1c levels showed 2.18 (95% CI 1.295 – 3.678) times higher chances of maternal hypertension. After controlling for pregnancy BMI (Kg/m^2), maternal age, hypertension prior to pregnancy, and GDM, this association becoming statistically insignificant [1.44 (95% CI 0.853 – 2.442)].

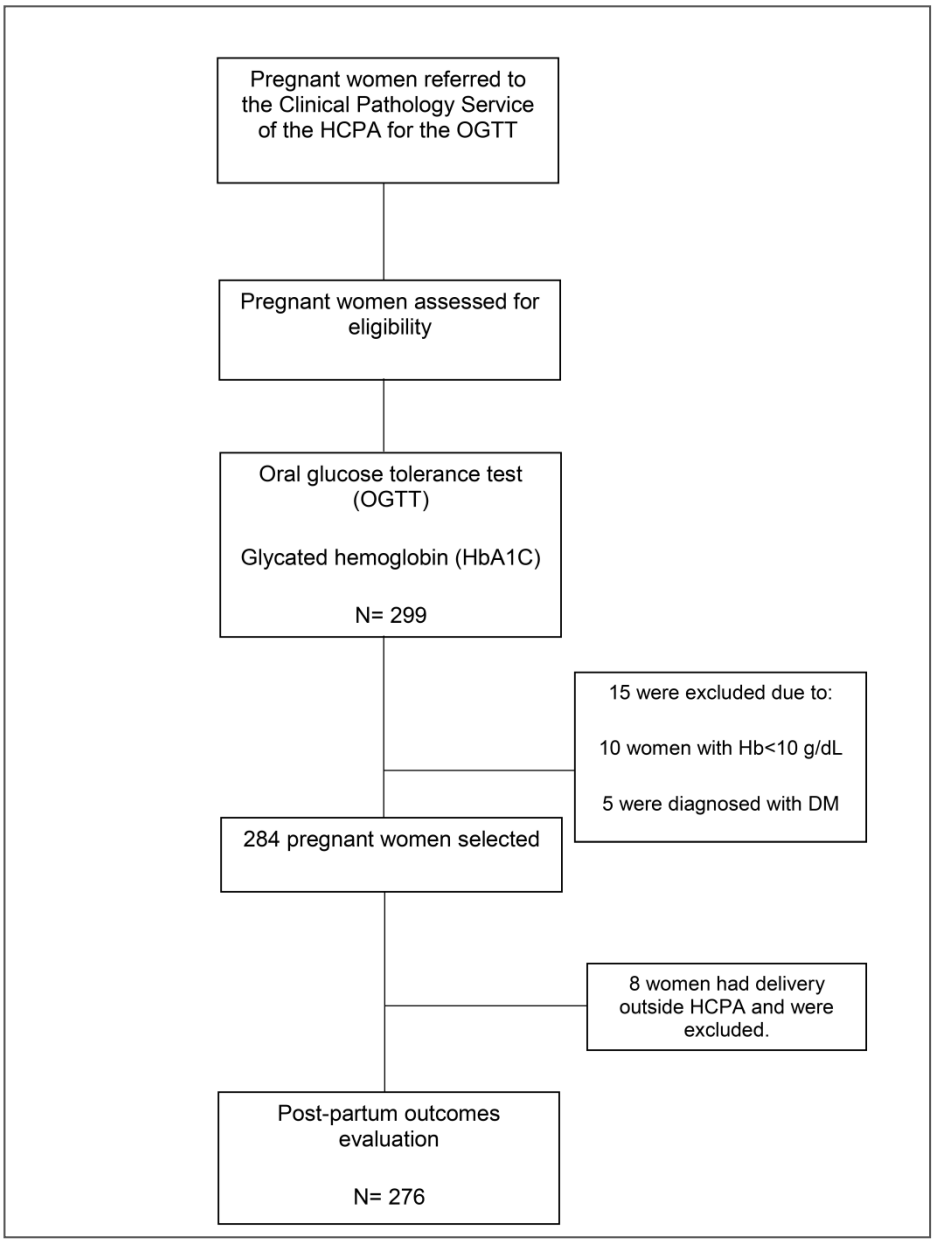

Figure1. Flowchart of the study

Table1 - Clinical and laboratory characteristics of the pregnant women with and without GDM by OGTT in this study

	- GDM	**+ GDM**	
	N = 201	N = 75	**P**
Age (years)	28.6 (±6.28)	31.4 (±6.02)	0.001
Gestational week (weeks)	26.4 (±4.51)	26.0 (±5.58)	0.431
BMI (Kg/m^2)	29.2 (±5.65)	33.2 (±6.44)	<0.001
Fasting glucose (mg/dL)	80.6 (±7.53)	93.1 (±10.99)	<0.001
1h glucose (mg/dL)*	120.1 (±25.84)	173.5 (±27.52)	<0.001
2h glucose (mg/dL)	107.4 (±19.95)	149.9 (±23.99)	<0.001
Hb (g/dL)	11.7 (±0.90)	11.9 (±0.83)	0.040
HbA1c (%, mmol/L)	5.0 (±0.39)	5.4 (±0.47)	<0.001

*****1h glucose was not available for 88 women.

Table 2 - Maternal and neonatal outcomes according to de HbA1c levels*.

	HbA1c ≤4.9% N = 89	**HbA1c 5.0–5.3** N = 108	**HbA1c ≥5.4** N = 79	**P**
Gestational age (weeks)	38.6 (2.04)	38.3(2.16)	37.9(1.84)	0.100
Cesarean delivery (n, %)	49 (55)	54 (50)	52 (66)	0.095
Hipertensive maternal outcomes (n, %)	16 (18)	32 (30)	31 (39)	**0.036**
Hypertension (n, %)	11 (12.4)	27 (25.0)	26 (32.9)	**0.006**
Preeclampsia (n, %)	6 (6.7)	8 (7.4)	7 (8.9)	0.870
Gestational diabetes mellitus (n, %)	9 (10.1)	24 (22.2)	42 (53.2)	**0.000**
Preterm delivery (n, %)	14 (15.7)	22 (20.4)	20 (25.3)	0.304
Birthweight (g)	3,202.4 (593.3)	3,102.4 (609.3)	3,083.5 (662.2)	0.393
Macrosomia (n, %)	4 (4.5)	7 (6.5)	5 (6.3)	0.815
Neonatal morbidity (n, %)	20 (22.5)	23 (21.3)	26 (32.9)	0.155
ICU hospitalization** (n, %)	12 (13.5)	15 (13.9)	13 (16.5)	0.839

*HbA1c quartiles, where interval of HbA1c 5.0-5.3% represents second and third quartile together.
ICU = intensive care unit; **ICU for any cause.

Table 3 - Association between HbA1c levels and adverse pregnancy outcomes.

	UOR (95% CI)	P	AOR* (95% CI)	P
Cesarean delivery				
HbA1c ≥5.4	1.196 (0.935 – 1.529)	0.155	1.148 (0.867 - 1.519)	0.335
HbA1c 5.0–5.3	0.910 (0.696 – 1.185)	0.478	0.868 (0.652 - 1,155)	0.331
HbA1c ≤4.9%	1		1	
Hypertensive outcomes of pregnancy				
HbA1c ≥5.4	2.18 (1.295 - 3.678)	**0.003**	1.44 (0.853 – 2.442)	0.172
HbA1c 5.0–5.3	1.65 (0.970 - 2.801)	0.065	1.56 (0.966 – 2.516)	0.069
HbA1c ≤4.9%	1		1	
Macrosomia				
HbA1c ≥5.4	1.48 (0.392 – 5.062)	0.600	1.57 (0.306 – 8.091)	0.588
HbA1c 5.0–5.3	1.44 (0.436 – 4.769)	0.549	1.50 (0.394 – 5.728)	0.552
HbA1c ≤4.9%	1		1	
Neonatal morbidity				
HbA1c ≥5,4	1.47 (0.890 – 2.410)	0.133	1.008(0.532 – 1.907)	0.982
HbA1c 5,0–5,3	0.95 (0.558 – 1.609)	0.842	0.82 (0.464 – 1.437)	0.483
HbA1c ≤4,9%	1		1	
ICU hospitalization				
HbA1c ≥5,4	1.22 (0.592 – 2.517)	0.590	0.904(0.382 – 2.143)	0.819
HbA1c 5,0–5,3	1.03 (0.509 – 2.085)	0.934	0.920(0.445 – 1.898)	0.821
HbA1c ≤4,9%	1		1	

*Adjusted for pregnancy BMI (Kg/m^2), maternal age, hypertension prior to pregnancy and GDM. UOR, unadjusted odds ratio; AOR, adjusted odds ratio. CI, confidence interval.

Discussion

This study aimed to evaluate the association of HbA1c levels with adverse maternal-fetal outcomes. Our results showed that HbA1c levels are associated with the presence of GDM and with hypertensive outcomes of pregnancy in the mother, however GDM has been shown to occur more frequently in women with chronic hypertension (RR 2.03) (14), but after adjustment for the presence of hypertension previously to pregnancy, relative risk (RR) for hypertensive outcomes of pregnancy was no statistically significant. Pregnancy-induced hypertension, however, was not a significant and independent predictor for GDM in the Yang et.al study (15). There was no association with HbA1c levels and adverse outcomes in the neonates, although we observed an increased rate of events in the higher quartiles of HbA1c for preterm-delivery, small babies for gestational age, and neonatal morbidity. We did not observe any association of HbA1c with macrossomia. In the Khalafalah et.al study by association of HbA1c levels with pregnancy or type of delivery complications was not observed (16). A systematic review about Gestational diabetes and pregnancy outcomes by Wendland et.al, demonstrated in your summary estimates of relative risk demonstrate that GDM diagnostic criteria based on both the WHO and the IADPSG criteria predict perinatal and maternal adverse outcomes. The strength of the crude associations found ranged from 1.23 (95% CI 1.01-1.51) for cesarean delivery, to 1.81 (95% CI 1.47-2.22) for macrosomia, but this crude associations are very small within a diagnostic context (17).

Several studies have analyzed these associations between HbA1c levels and pregnancy outcomes (8,18,19,20,21). However, these associations are significant in univariate analysis, after adjustment for confounder factors several of these

associations did not remain significant, similar to our findings. Interesting, several of these studies analyzed these associations only in pregnant women affected by GDM.

Bhavadharini and colleagues reported in their study a significant increase in the occurrence of macrosomia in pregnant women with HbA1c values ≥ 5.0%, however macrosomia classification used by these authors was of neonates weighing 3.5 kg or greater (19). In our study, we defined macrosomia as neonates weighing 4.0 kg or greater. Although we found an increase in risk for the development of this outcome, after adjustment for possible confounders this increased risk did not remain significant (19).

Mañé and colleagues studied a multiethnic cohort and observed that HbA1c ≥5.9% was independently associated with a 3-fold increased risk of macrosomia and preeclampsia when HbA1c was measured in the first trimester of pregnancy, even after adjusting for potential confounders. However, there were no statistically significant differences in other pregnancy outcomes (22).

The strengths of our study were: first we have evaluated all women at the third trimester of pregnancy with an OGTT. We also measured HbA1c in all women at the same moment with an standardized method and excluded possible interfering factors in its measurement. The sample size was calculated to guarantee the statistically power of analysis. Our study has limitations. The cohort studied here was small and we have a small rate of adverse events what would account for associations with low statistically power. In addition, our patients were recruited in a tertiary hospital and may represent a subgroup of patients at high risk of adverse outcomes and consequently at a more restrict clinical attention.

In conclusion, HbA1c levels are associated with hypertensive outcomes of pregnancy, however this association is not significant when adjusted for pregnancy

BMI (Kg/m^2), maternal age, hypertension prior to pregnancy and GDM. Our results did not show association of HbA1c levels and other maternal-fetal adverse outcomes.

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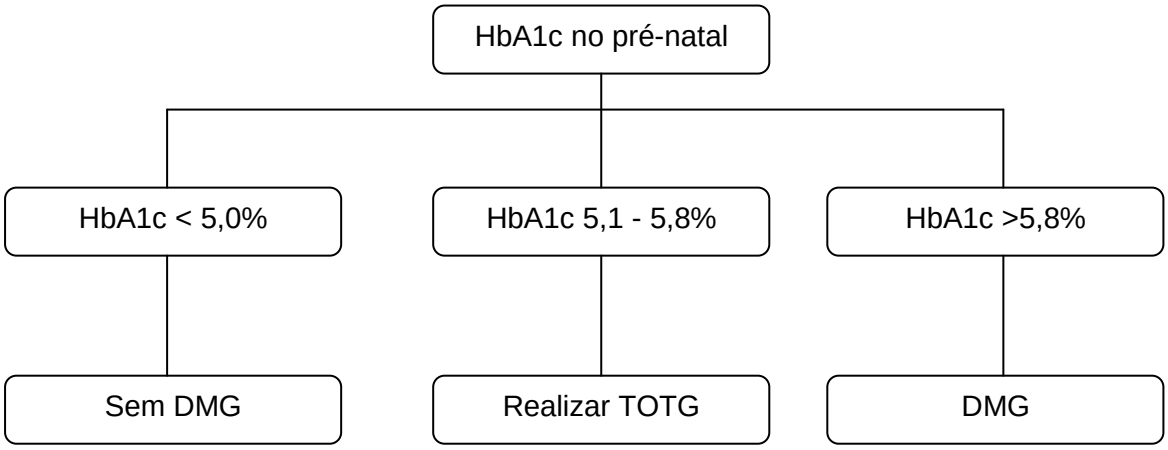
8. CONCLUSÕES

Esta tese teve como uns dos objetivos avaliar a possível interferência da suplementação de ferro durante a gestação nos níveis de HbA1c. Os resultados desse estudo demonstraram que a suplementação de ferro durante a gravidez não afeta os níveis de HbA1c e não tem impacto clínico na interpretação final dos resultados na ausência de anemia ou presença de anemia leve, no entanto, em mulheres com anemia moderada ou grave em suplementação de ferro esse teste pode sofrer alteração, concordando com outros estudos que demonstraram que os valores de HbA1c dependem dos níveis de anemia do paciente.

Também tivemos como objetivo avaliar a utilização da HbA1c no diagnóstico do DMG através de uma revisão sistemática e metanálise dos estudos disponíveis na literatura. Nossos resultados da metanálise mostraram que a partir do ponto de corte de HbA1c 5,8% tem-se especificidade suficiente para a confirmação do diagnóstico de DMG. Com base nos dados dessa metanálise propusemos o seguinte algoritmo para o rastreamento dessa doença:



Se aplicássemos o algoritmo proposto, utilizando os dados do estudo de Renz 2015, ao realizarmos a HbA1c no pré-natal nas 262 gestantes teríamos 66 gestantes

sem diagnóstico de DMG (HbA1c< 5,0%) que seguiriam com os cuidados normais do pré-natal, 32 gestantes com DMG (HbA1c >5,8%) que passariam a tratar a doença e apenas 164 gestantes que tiveram resultados de HbA1c entre 5,1 e 5,8% seriam submetidas ao TOTG, uma redução de 37% de gestantes a realizar esse teste. Também é importante ressaltar que a utitização do TOTG como teste de referência no diagnóstico do DMG, especialmente quando utilizados os critérios propostos pela IADPSG, identifica menores graus de hiperglicemia.

Ao avaliarmos a associação da HbA1c, utilizada como teste diagnóstico para DMG, com desfechos materno-fetais, encontramos um aumento significativo na ocorrência de desfechos hipertensivos da gestação relacionada com o aumento nos níveis de HbA1c. Apesar de encontrarmos fortes razões para a utilização da HbA1c no diagnóstico de DMG, são necessários mais estudos para que se consiga aumentar a força estatística para confirmar as associações com alguns desfechos gestacionais.

Além da HbA1c como alternativa ao TOTG, temos a albumina glicada (AG), teste laboratorial que tem ganhado destaque como um marcador para o monitoramento glicêmico no DM. Por não ser afetada pela presença de processos hemolíticos e de Hb anormais, a AG poderia ser utilizada como marcador glicêmico no período gestacional.

Considerando impacto das complicações dessa doença, a dosagem da hemoglobina glicada torna-se um importante procedimento nos laboratórios clínicos. Porém é necessário que os laboratórios clínicos participem ativa e regularmente de proficiência específico para a hemoglobina glicada e utilizem os métodos certificados

pelo NGSP (*National Glycohemoglobin Standardization Program*) e do estudo do *Diabetes Control and Complications Trial* (DCCT).