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Programa de Pós-graduação em Ciências Médicas: Endocrinologia

Albumina glicada como marcador glicêmico em diferentes contextos clínicos

TESE DE DOUTORADO

Fernando Chimela Chume

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Orientadora: Profª Drª Joíza Lins Camargo

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FORMATO DA TESE DE DOUTORADO

Esta tese de Doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, sendo apresentada através de uma fundamentação teórica e quatro manuscritos originais acerca do tema estudado:

1) Revisão sistemática com meta-análise sobre a acurácia diagnóstica do teste de albumina glicada no diagnóstico de diabetes mellitus na população geral:

   "Glycated albumin in diabetes mellitus: a meta-analysis of diagnostic test accuracy" (publicado em 27 de abril de 2022; https://doi.org/10.1515/cclm-2022-0105)

2) Avaliação do desempenho da albumina glicada no momento da admissão para detectar anormalidades glicêmicas em indivíduos hospitalizados pela doença do coronavírus 2019 (COVID-19):

   "Performance of Admission Glycated Albumin in the Detection of Glycaemic Abnormalities During COVID-19 Hospitalization"

3) Avaliação da acurácia diagnóstica da albumina glicada no diabetes mellitus gestacional utilizando o teste oral de tolerância à glicose como método de referência:

   "Is there a role for glycated albumin in the diagnosis of gestational diabetes mellitus?" (publicado em 14 de março de 2021; https://doi.org/10.1007/s12020-021-02673-6)

4) Análise da relação do estado glicêmico definido por teste oral de tolerância à glicose, níveis de HbA1c e albumina glicada com desfechos adversos da gravidez em gestantes com e sem diabetes mellitus gestacional (DMG):

   "Relationship of glycaemic status by oral glucose tolerance test, HbA1c and glycated albumin levels with adverse pregnancy outcomes"

DEDICATÓRIA

À minha família.

"*Families are our Global Positioning System (GPS). They guide us to reach great heights and support us when trouble occasionally comes.*"

~Fernando Chimela Chume

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LISTA DE ABREVIATURAS PARA A INTRODUÇÃO

ADA – American Diabetes Association

AG – Albumina glicada

AGEs – Advanced glycated end products

ANVISA – Agência Nacional de Vigilância Sanitária

ARIC – Atherosclerosis Risk in Communities

AUC – área sob a curva

CGM – Monitoramento contínuo de glicose

COVID-19 – doença do coronavírus 2019

DCCT – The Diabetes Control and Complications Trial Research Group

DMG – Diabetes mellitus gestacional

DMPT – diabetes mellitus pós-transplante

DOR – *Diagnostic odds ratio*

DRC – Doença renal crônica

ELISA – Ensaio de imunoabsorção enzimática

GJ – Glicemia de jejum

GPF – Fragmentos de proteína glicada

GSP – *Glycated serum proteins*

HbA1c - Hemoglobina glicada

HIV – Vírus da imunodeficiência humana

HPLC – Cromatografia líquida de alta eficiência

H_2O_2 – Peróxido de Hidrogênio

IC – Intervalo de confiança

ICAM-1 – Molécula de adesão intercelular-1

NF-*k*B - Fator de transcrição nuclear kappa de célula B

PF – Fragmentos de proteína

r – coeficiente de correlação

RAGE –*Receptor of Advanced Glycation Endproduct*

SARS-CoV-2 – coronavírus da síndrome respiratória aguda grave 2

TOTG – Teste oral de tolerância à glicose

VCAM-1 – Molécula de adesão celular vascular -1

2hPG - Glicemia de duas horas pós sobrecarga de 75g de glicose

RESUMO

Diabetes mellitus é um preocupante problema de saúde pública e os marcadores glicêmicos são importantes para o seu diagnóstico e tratamento. Na rotina clínica, glicose plasmática e hemoglobina glicada (HbA1c) são medidas usadas para rastrear, diagnosticar e manejar o diabetes, mas ambas têm limitações. A glicose plasmática pode ser afetada pelo jejum, ingestão de alimentos, estresse agudo, e é susceptível a variabilidade intrapessoal e às interferências pré-analíticas enquanto HbA1c não é adequada em condições com meia-vida das hemácias alterada, como anemia e doença renal crônica.

Como a albumina glicada (AG) supera as principais limitações apresentadas pela glicose plasmática e HbA1c, há um interesse crescente em utilizá-la como teste alternativo ou complementar para o rastreio, diagnóstico e manejo de diabetes. AG reflete a concentração média de glicemia nas últimas 2–3 semanas e é usada na prática clínica em alguns países da Ásia. No entanto, a utilidade da AG em diversas condições clínicas permanece incerta e os pontos de corte diagnósticos da AG na população geral não foram estabelecidos. Nesta tese realizamos uma revisão sistemática com meta-análise para avaliar o desempenho da AG no diagnóstico de diabetes mellitus na população em geral, avaliamos o desempenho da AG no momento da admissão para detectar anormalidades glicêmicas em indivíduos hospitalizados pela doença do coronavírus 2019 (COVID-19), avaliamos a acurácia diagnóstica da AG na detecção do diabetes mellitus gestacional (DMG), e analisamos também a relação do estado glicêmico definido por teste oral de tolerância à glicose (TOTG), níveis de HbA1c e AG com desfechos adversos da gravidez em gestantes com e sem DMG.

Na meta-ánalise, a AG apresentou boa acurácia diagnostica para diabetes, e AG ≥17,1% mostrou alta especificidade para detectar diabetes, com poucos casos falso-positivos. Durante a hospitalização por COVID-19, AG apresentou acurácia moderada à ótima nas condições estudadas. Os pontos de corte de AG no momento da admissão de 19,0%, 21,0% e 20,0% apresentaram alta especificidade para identificar diabetes prévio não diagnosticado pré-admissão, diabetes não controlado e hiperglicemia intra-hospitalar que necessitou de prescrição de insulina, respectivamente. No entanto, AG não foi precisa na identificação de hiperglicemia no momento da admissão em indivíduos sem evidência de diabetes

prévia. Em gestantes, AG na 24ª–32ª semana de gestação apresentou baixa sensibilidade para DMG, sem capacidade de discriminar gestantes com e sem DMG. Além disso, apresentou baixo valor preditivo de risco para desfechos perinatais adversos em gestantes com e sem DMG.

Com base nos resultados dos estudos que compõem a presente tese, concluímos que: *(1)* AG é um marcador glicêmico útil e com desempenho adequado para o diagnóstico de diabetes na população geral; *(2)* AG também pode ser utilizado no momento da admissão para identificar adultos com diabetes prévio não diagnosticado pré-admissão, diabetes não controlado e hiperglicemia intra-hospitalar que necessitou de prescrição de insulina durante a hospitalização por COVID-19; *(3)* o teste AG na 24ª–32ª semana de gestação é incapaz de identificar gestantes com DMG assim como predizer gestantes com risco aumentado de desfechos perinatais adversos.

Descritores: Albumina glicada; Acurácia diagnóstica; Controle glicêmico; Diabetes; diabetes mellitus gestacional; COVID-19.

ABSTRACT

Diabetes is an alarming public health problem and glycaemic markers are essential for its diagnosis and treatment. In clinical routine, plasma glucose and glycated haemoglobin (HbA1c) are used to screen, diagnose, and management of diabetes, but both have limitations. Plasma glucose can be affected by fasting, food intake, acute stress, and is susceptible to intra-individual variability and preanalytical interferences, whereas HbA1c is not suitable for conditions with altered red blood cell turnover, such as anaemia and chronic kidney disease.

As glycated albumin (GA) overcomes the main limitations presented by plasma glucose and HbA1c, there is a growing interest in using GA as an alternative or complementary test for the screening, diagnosis, and management of diabetes. GA reflects the average blood glucose concentration over the past 2–3 weeks and is used in clinical practice in some Asian countries. However, the usefulness of GA in various clinical conditions remains uncertain, and diagnostic cut-offs for GA in the general population have not been established. In this thesis, we performed a systematic review with meta-analysis to evaluate the performance of GA in the diagnosis of diabetes mellitus in the general population, we evaluated the performance of GA at the time of admission to detect glycemic abnormalities in individuals hospitalized with coronavirus disease 2019 (COVID-19), we evaluated the diagnostic accuracy of GA in the detection of gestational diabetes mellitus (GDM), and we also analyzed the relationship between glycaemic status defined by oral glucose tolerance test (OGTT), HbA1c and GA levels with adverse pregnancy outcomes in pregnant women with and without GDM.

In the meta-analysis, GA showed good diagnostic accuracy for diabetes, and AG ≥17.1% showed high specificity for detecting diabetes, with few false-positive cases. During hospitalization with COVID-19, GA showed moderate to optimal accuracy in the conditions studied. The GA cut-offs at admission of 19.0%, 21.0%, and 20.0% showed high specificity for identifying undiagnosed pre-admission diabetes, uncontrolled diabetes, and in-hospital hyperglycaemia requiring insulin prescription, respectively. However, GA was not accurate in identifying hyperglycaemia on admission in individuals without evidence of previous diabetes. In pregnant women, GA at the 24th–32nd week of pregnancy showed low sensitivity

for GDM, with no ability to discriminate pregnant women with and without GDM. In addition, it had a low predictive value of risk for adverse perinatal outcomes in pregnant women with and without GDM.

According to these findings of the studies that make up this thesis, we conclude that: *(1)* GA is a useful glycaemic marker with adequate performance for diagnosing diabetes in the general population; *(2)* GA may also be used at the time of admission to identify adults with pre-admission undiagnosed diabetes, uncontrolled diabetes, and uncontrolled hyperglycaemias during hospitalization for COVID-19; *(3)* the GA test at the 24th–32nd week of gestation is unable to identify pregnant women with GDM as well as predict pregnant women at increased risk of adverse perinatal outcomes.

Key words: Glycated albumin; Diagnostic accuracy; Glycaemic control; Diabetes; Gestational diabetes mellitus; COVID-19.

SUMÁRIO

Capítulo 1

Albumina glicada como marcador glicêmico: bioquímica e prática clínica

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1. INTRODUÇÃO

Diabetes mellitus é um grupo heterogêneo de distúrbios metabólicos caracterizados por um aumento anormal dos níveis de glicose no sangue, decorrente da falta de insulina e/ou da incapacidade de a insulina exercer adequadamente seus efeitos. A hiperglicemia crônica pode causar lesões a longo prazo de vasos sanguíneos, nervos e órgãos em todo o corpo, provocando complicações graves, como insuficiência renal, perda de visão, acidente vascular cerebral, doença cardiovascular e desfechos adversos maternos e fetais. Em 2021, estimou-se que 21 milhões de nascidos vivos foram afetados por hiperglicemia na gestação e mais de 6,7 milhões de adultos morrerão de causas relacionadas ao diabetes, excluindo os riscos de mortalidade associados à pandemia de doença do coronavírus 2019 (COVID-19) [1].

Atualmente para estabelecer o diagnóstico de diabetes podem ser usados a glicemia de jejum (GJ), o teste de tolerância oral à glicose (TOTG) e a hemoglobina glicada (HbA1c). Os resultados são igualmente apropriados para testes de diagnóstico, apesar de não necessariamente detectarem o diabetes nos mesmos indivíduos [1–4]. No entanto, a medição do TOTG não tem reprodutibilidade, é demorada e requer no mínimo duas amostras de sangue. Além disso, assim como a GJ, o TOTG também exige jejum, reduzindo seu uso para triagem aleatória. Ambos os testes podem ser afetados por estresse causado por uma doença aguda. Também, os testes dependem de manuseio rigoroso da amostra coletada para evitar a glicólise antes da centrifugação e interpretação inadequada dos resultados [5,6].

A HbA1c é o mais recente marcador glicêmico a ser incluído nos critérios diagnósticos do diabetes [7, 8] e é comumente referido como o teste de escolha por não requerer jejum e não ser afetado por hiperglicemia de estresse em condição aguda. No entanto, a HbA1c é uma medida indireta dos níveis médios de glicose no sangue e a relação da sua concentração com níveis médios de glicemia é altamente dependente do tempo de meia vida dos eritrócitos. Devido a isso, HbA1c não é adequada em condições com tempo de vida das hemácias alterado, como em transfusões recentes e aumento de eritropoiese secundária à hemólise, perda de sangue, bem como doação de sangue recente [2, 4, 9, 10]. Em pessoas

com doença renal crônica (DRC) em estágio terminal, além da anemia, transfusões recentes e uso de eritropoetina que resultam em valores reduzidos de HbA1c, pode haver interferência causada pela hemoglobina carbamilada [9]. A anemia por deficiência de ferro, um importante problema de saúde pública nos países em desenvolvimento, está associada ao aumento da glicação da hemoglobina e consequentemente valores aumentados de HbA1c [9, 11]. Outras condições como gravidez, fibrose cística, hemodiálise e variantes genéticas de hemoglobina podem afetar a glicação da hemoglobina independentemente da glicemia e diminuir a acurácia da HbA1c [2, 4, 9, 10]. Além disso, a HbA1c apresenta baixa sensibilidade e alta especificidade em identificar diabetes diagnosticado pelo critério de TOTG. Dados brasileiros também corroboram essa informação [12, 13]. Na verdade, HbA1c ≥6,5% (48 mmol/mol) diagnosticam apenas 30% dos casos de diabetes identificados coletivamente usando HbA1c, GJ e/ou glicemia de duas horas pós sobrecarga de 75g de glicose (2hPG) [14]. Também, é sugerido que os níveis de HbA1c sofrem influência das etnias, idade e tratamento do HIV (vírus da imunodeficiência humana) independentemente da glicemia [2, 15–17].

Os desafios acima justificam o interesse crescente em um marcador glicêmico que possa minimizar as limitações dos testes de glicose, mas também ser independente dos eritrócitos. Nesse sentido, a albumina glicada (AG), parece ser um teste candidato. AG é produzida por uma reação não enzimatica entre glicose e albumina [18] e pode ser medida por ensaios enzimáticos em equipamentos automatizados. AG é independente de hemoglobina/eritrócitos e reflete a concentração média de glicosse nas últimas 2–3 semanas, tempo médio de vida da albumina, em vez de 2–3 meses observados com HbA1c [19, 20]. Na população geral, o desempenho da AG no diagnóstico de diabetes é semelhante ao da HbA1c [21–31] sugerindo que seu uso pode ser uma alternativa à HbA1c em condições em que este último não reflete o status glicêmico com precisão [21–31]. Além disso, a AG, com valores preditivos semelhantees à HbA1c, foi associada a complicações diabéticas e mortalidade no paciente com diabetes [32–36].

Embora a AG tenha potencial para uso no diagnóstico de diabetes e no acompanhamento do controle glicêmico em pessoas com diabetes, deve-se ressaltar que ainda não existe consenso dos pontos de corte da AG para o

diagnóstico de diabetes e prognóstico de complicações do diabetes. Além disso, existem poucos estudos em outras populações, inclusive em gestantes e em indivíduos com estresse induzido por uma condição aguda como COVID-19.

O objetivo desta revisão é resumir os estudos existentes sobre AG, descrevendo suas propriedades bioquímicas, os efeitos da glicação, as implicações patológicas dos altos níveis de AG, os métodos de quantificação de AG e o uso de AG como biomarcador complementar para diagnóstico de diabetes e monitoramento de desfechos adversos.

2. ALBUMINA GLICADA

2.1. Albumina, sua glicação e impactos fisiopatológicos

A albumina sérica humana é a mais abundante proteína circulante com a concentração normal entre 3,5 e 5,0 g/dL e representa cerca de 50% das proteínas totais do soro humano [37]. É sintetizada pelo fígado e rapidamente excretada a uma taxa de cerca de 10 g a 15 g por dia na corrente sanguínea, onde exerce suas funções fisiológicas [38]. Comparada a outras proteínas, a albumina é uma molécula relativamente pequena, com um peso molecular em torno de 67000 Daltons [37, 38]. Uma das importantes funções da albumina é o seu papel na manutenção da pressão oncótica, devido ao seu peso molecular relativamente baixo, à sua alta concentração, e pelo seu ponto isoelétrico fraco com carga global negativa em pH fisiológico [37]. A albumina desempenha também, um papel na manutenção do equilíbrio acidobásico. Na estrutura de albumina tem resíduos de histidina, que por terem um pKa baixo conferem a albumina uma função de tamponamento em situações de acidose metabólica, assim como em alcalose metabólica com liberação dos seus íons hidrogênio [37, 39, 40]. Além disso, existe uma cisteína livre na posição 34, que atribui à albumina uma função de antioxidante fisiológico [41, 42]. No entanto, a modificação química da albumina induzida por processos enzimáticos e não enzimáticos, incluindo glicação, oxidação, altera as funções biológicas da proteína [43]. Além da albumina, propriedades estruturais e funcionais de várias proteínas, como hemoglobina, lipoproteína são afetadas pela glicação [44].

O termo glicação é utilizado para designar a reação espontânea não enzimática de um carboidrato ou seu produto de degradação com grupo amino livre de proteína. Assim como a formação dos demais produtos glicados, a formação de albumina glicada é bastante complexa, mas três grandes etapas são distinguíveis: inicial, intermediária e a final. Na etapa inicial, ocorre a interação do grupo amino da albumina e grupo carbonila do monossacarídeo redutor acíclico que resulta na formação de uma aldimina intermediária. Este produto é instável e é conhecido como base de Schiff. A etapa intermediária envolve a transformação química (rearranjos de Amadori) da base de Schiff em uma cetoamina mais estável (produto de Amadori). A última etapa trata das reações de clivagem não oxidativa, degradação oxidativa e reações de reticulação dos produtos Amadori, levando a

formação de produtos finais de glicação avançada (AGEs) irreversíveis. A descrição detalhada do processo de glicação está disponível nas revisões de Cho et al [45]. Eles sugerem mais etapas do processo de glicação, mas também a possibilidade de produzir os mesmos AGEs por diferentes vias químicas. Isso pode explicar parcialmente a dificuldade de achar uma única estratégia para a prevenção e manejo da formação de AGE, que é considerada uma das principais causas de várias complicações.

A albumina sérica humana é altamente sensível à glicação, principalmente por sua meia vida, alta concentração, e pelo grande número de resíduos de lisina, arginina e cisteína que podem participar na glicação [46]. Embora existam vários resíduos de lisina e arginina na estrutura da albumina, poucos são susceptíveis a glicação. O principal local de glicação da albumina é lisina 525 [47-50]. É importante mencionar que a extensão da glicação da albumina depende fortemente da duração da exposição da albumina a altos níveis de concentração de carboidratos redutores (glicose) [51]. E essa reatividade da albumina a níveis de glucose é uma das razões que tornam a AG um biomarcador interessante para o controle glicêmico.

Na forma glicada, a albumina não apresenta apenas alterações em suas funções fisiológicas já citadas acima, mas também adquire um fenótipo patológico. Níveis elevados de AG podem causar danos irreversíveis de vários órgãos e tecidos. Por exemplo, no rim, a AG é transportado através dos capilares glomerulares e absorvido pelas células epiteliais e mesangiais, onde aumenta a produção de moléculas pró-oxidantes e contribui para o aparecimento da doença renal do diabetes [52–54]. Nas doenças cardiovasculares, a AG desempenha um papel na ativação e agregação de plaquetas, promovendo a oxidação e a expressão de moléculas de adesão, incluindo a molécula de adesão intercelular-1 (ICAM-1) e a molécula de adesão celular vascular (VCAM-1), envolvidas na formação da lesão aterosclerótica [37, 55–57]. Na verdade, esse processo resulta da interação dos AGEs derivado da glicação da albumina com receptores de superfície celular para AGE *(*RAGE – *“Receptor Advanced Glycation Endproducts”)*. A interação AGE-RAGE culmina na ativação do fator de transcrição nuclear kappa de célula B (NF-κB). O NF-κB induz a produção de citocinas pró-inflamatórias e fatores de crescimento, apoptose, estresse oxidativo e atividades

pró-trombóticas, eventos que têm sido associados a consequências patológicas de aumento dos níveis AGE e AG [37, 51, 58]. Efeito similar ao da AG no endotélio para desenvolver as complicações ateroscleróticas ocorre nas células micróglias na retinopatia [37, 51].

Em concordância com as patogêneses acima indicadas, vários estudos indicam existir uma associação independente entre AG e as complicações crônicas do diabetes. Níveis elevados de AG associam-se independentemente com complicações microvasculares e macrovasculares do diabetes [33–35, 59–62]. Esses achados sugerem o uso de AG como um marcador glicêmico com poder preditor de efeitos adversos do diabetes.

2.2. Aspectos laboratoriais da AG

2.2.1. Métodos laboratoriais

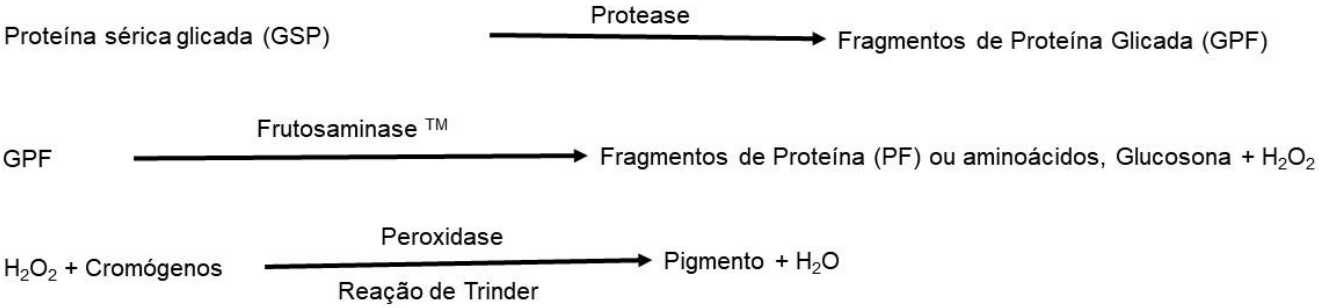
Existem vários métodos de quantificação de AG, entre eles a cromatografia líquida de alta eficiência (HPLC); cromatografia de afinidade; técnicas de imunoensaio, incluindo também a quantificação por radioimunoensaio, imunoenzimático (ELISA); colorimétrica com ácido tiobarbitúrico e eletroquímica [18, 37]. No entanto, esses métodos atualmente não estão disponíveis na rotina laboratorial, pois apresentam muitas desvantagens devido à complexidade da técnica, ao alto custo, baixa precisão do método [18].

Atualmente existem vários ensaios enzimáticos precisos, que foram desenvolvidos com base na protease específica para AG [18]. Esses ensaios são fáceis de usar e podem ser automatizados, o que os torna adequado para fins clínicos. Hoje o ensaio mais amplamente avaliado em pesquisas clínicas é conhecido como *Lucica GA-L (Asahi Kasei Pharma, Tokyo, Japan)* [63]. *Lucica GA-L* é amplamente utilizado em rotina no Japão, China, Taiwan e Coréia como marcador glicêmico, mas ainda não foi aprovado pela Agência Nacional de Vigilância Sanitária (ANVISA) para uso no Brasil. Todavia, outro teste com princípio similar ao *Lucica* GA-L e aprovado pela ANVISA para a dosagem de AG em pesquisas clínicas é o *Diazyme GSP (GlycoGap®, Diazyme Laboratories, Poway, CA)*. O ensaio de *Diazyme* GSP usa uma protease específica para converter proteínas séricas glicadas (GSP, de *glycated serum protein*) em fragmentos de proteína glicada (GPF) de baixo peso molecular. Frutosaminase específica de

Diazyme catalisa a reação oxidativa do produto Amadori de GPF para produzir fragmentos de proteína (PF) ou aminoácidos, glucosona e H_2O_2. O H_2O_2 liberado é medido por uma reação colorimétrica de Trinder. A absorbância gerada em 546 nm é proporcional à concentração de GSP na amostra.

No geral, o ensaio de *Diazyme* GSP tem excelente reprodutibilidade e na sua comparação com *Lucica GA-L* apresentou uma excelente correlação [64, 65]. Para esta comparação, os valores de GSP foram determinados com o ensaio de *Diazyme GSP* e os valores totais de albumina com o método de bromocresol verde (BCG, de *Bromocresol Green*). Os valores de GSP obtidos foram convertidos para % AG usando uma equação de conversão. A figura abaixo mostra o princípio do ensaio de *Diazyme GSP*, de albumina e o cálculo de AG.

Passo 1: Ensaio de *Diazyme GSP*



Passo 2: Ensaio de albumina

Passo 3: Cálculo da percentagem de albumina glicada (AG)

$$AG(\%) = \frac{\text{GSP } (\mu\text{mol/L}) \text{ x } 0{,}182 \ + \ 1{,}97}{\text{Albumina total } (\text{g/d L})} + 2{,}9$$

Figura 1. Princípio do teste de AG usando ensaio de *Diazyme GSP*.

Os valores de AG medidos pelo ensaio de *Diazyme GSP* foram significativamente correlacionados com os valores de AG medidos pelo ensaio de *Lucica GA-L* (r^2 = 0.975) [64]. E mais, acredita-se que os valores de AG expressos

em percentual são mais compreensíveis na prática clínica do que expressos GSP em unidades do sistema internacional µmol/L [65].

2.2.2. Preparo para o exame

A dosagem de AG pelo método enzimático pode ser feita em amostras de plasma ou soro com precisão similar [23]. Desse modo, a AG pode ser analisada juntamente com marcadores biológicos comuns sem requerer uma coleta de sangue em um tubo separado. Além disso, não é necessário jejum para a coleta do material. Os valores de AG plasmática e sérica não sofrem alterações quando avaliados em amostras coletadas em jejum, pós-prandial ou mesmo após sobrecarga com glicose [18, 23]. O ensaio demostra ser muito estável mesmo em amostras de soro congeladas e armazenadas por um longo período de tempo (19 – 23 anos a –70ºC) [66].

3. APLICAÇÃO CLÍNICA DA ALBUMINA GLICADA (AG)

3.1. AG para rastreamento e diagnóstico de diabetes mellitus

A AG é atualmente utilizada em países asiáticos, para rastreamento de diabetes, classificação populacional e estratificação do risco de desenvolver diabetes, e para a condução terapêutica de indivíduos com diabetes [30, 67–69]. Nos estudos de acurácia diagnóstico realizados na população geral foi demostrado um desempenho bom a excelente da AG no diagnóstico de diabetes, com áreas sob as curvas (AUC) variando de 0,70 a 0,95 [21–31, 70]. Nesses estudos, os pontos de corte ótimos de AG para detectar diabetes variaram principalmente entre 15% e 18%, independentemente dos padrões de referência de diagnóstico de diabetes ou dos ensaios de AG utilizados. No entanto, esses pontos de corte ótimos de AG apresentavam uma especificidade quase perfeita, mas sensibilidade baixa a moderada para detectar diabetes [21–31, 70]. No geral, o desempenho visto na AG no diagnóstico de diabetes tem similaridade com o da HbA1c. No entanto, a AG não detecta necessariamente os mesmos indivíduos identificados por HbA1c [26, 71-73].

Estes dados sustentam que AG pode ser um marcador adicional útil não só para o rastreio e diagnóstico de diabetes, mas também em estratificação de risco. Em uma revisão sistemática e metanálise que avaliou a acurácia da AG, o ponto

de corte de 14,0% apresentou uma estimativa sumária de sensibilidade de 0,766, especificidade de 0,687, AUC de 0,80 e DOR de 7,176 [74]. No entanto, os dados avaliados nesta metanálise incluíram amostras de diferentes populações como receptores de transplante renal [75] e indivíduos com idade de 10 a 18 anos com fibrose cística [76]. Portanto, ainda faltam estudos com maior nível de evidência, que resumam as evidências sobre a acurácia da AG no diagnóstico de diabetes em população geral.

3.2. AG para rastreamento e diagnóstico de diabetes em população específica

O TOTG é o exame laboratorial ideal para o diagnóstico de diabetes em populações especificas como gestantes ou receptores de transplante renal. Porém, durante a pandemia de COVID-19 ficou mais evidente que seu uso rotineiro não é uma realidade na prática clínica, onde os testes de glicemia de jejum e/ou HbA1c são mais utilizados ou recomendados para o rastreamento e diagnóstico de diabetes em geral assim como em diabetes mellitus gestacional (DMG) e diabetes mellitus pós transplante renal (DMPT) [31, 70, 77, 78]. HbA1c na admissão também é recomendado em pacientes hospitalizados para identificar diabetes não controlado assim como diabetes prévio não diagnosticado antes da admissão [79]. Entretanto, como na população geral, devido à baixa sensibilidade da HbA1c não é considerado um método ideal de rastreamento e diagnóstico do DMG e DMPT nessas populações [2, 80–82]. Dois estudos que avaliaram desempenho de AG para diagnóstico de DMG mostraram que há uma baixa capacidade do teste de discriminar gestantes com e sem DMG [82, 83]. Embora esses estudos desencorajem o uso de AG para o diagnóstico de DMG em gestantes, são necessários mais estudos de acurácia diagnóstica em diferentes populações para aumentar a coerência e a fiabilidade das conclusões.

Em receptores de transplante renal, apenas um estudo transversal realizado nos primeiros meses pós-transplante mostrou que AG apresentava uma acurácia diagnóstica moderada para DMPT por TOTG e/ou HbA1c, com AUC de 0,67 a 0,71 [84].

Uma outra importante lacuna da literatura disponível diz respeito ao desempenho de AG como marcador glicêmico em indivíduos hospitalizados com enfermidade aguda.

3.3. AG no controle glicêmico e na predição de desfechos adversos do diabetes

Sugere-se existir uma associação entre AG e o desenvolvimento das complicações crônicas do diabetes. O estudo de coorte *The Diabetes Control and Complications Trial Research Group* (DCCT) avaliou 497 indivíduos com diabetes tipo 1, acompanhados por 6,5 anos, e mostrou que AG e HbA1c estavam intimamente correlacionados entre si e com a glicemia média. Nas análises da coorte, a AG e HbA1c mostraram associações semelhantes com retinopatia e nefropatia. Essas associações foram fortalecidas quando ambos os testes foram considerados [35]. Dados da coorte prospectiva do estudo *Atherosclerosis Risk in Communities* (ARIC), que acompanhou 11.348 adultos sem diabetes e 958 adultos com diabetes (tipo 1 e 2) por duas décadas, também relataram que a AG e frutosamina estavam fortemente associados a complicações microvasculares [retinopatia e DRC], com valor prognóstico comparável ao HbA1c. Mas HbA1c superou AG e frutosamina para a predição da incidência de diabetes. No entanto, a AG foi altamente correlacionada com HbA1c e GJ [33]. Em outro estudo epidemiológico [85], uma análise transversal mostrou associações em forma de J para AG e HbA1c com DRC. Onde, valores muito baixos de AG e HbA1c em indivíduos sem diabetes foram modestamente associados à DRC, e níveis elevados de AG e HbA1c foram fortemente associados à DRC. Adultos com diabetes e controle glicêmico ruim (AG >17,7% ou HbA1c ≥7,0%) eram mais propensos a ter DRC. O uso da AG forneceu informações complementares à HbA1c em relação a prevalência de DRC [85]. Esses achados sugerem que AG, se disponível, pode ser usada como um marcador glicêmico adicional.

As evidências da associação entre AG e neuropatia ainda são limitadas. Estudo transversais mostraram associação entre AG e neuropatia periférica [86–89]. E, quando comparada com HbA1c, AG parece ser um marcador glicêmico com capacidade superior de detectar neuropatia periférica [88, 89].

Em estudos prospectivos, que acompanharam indivíduos com e sem diabetes por média 6 e 20 anos, as associações de AG com desfechos vasculares e mortalidade foram semelhantes às observadas para HbA1c. Nesses estudos observou-se uma correlação mais forte entre os marcadores glicêmicos e uma associação mais forte com os desfechos em indivíduos com diabetes, quando comparado com indivíduos sem diabetes [34, 36].

Em um estudo na comunidade japonesa com 2.965 participantes e acompanhamento médio de 10 anos, também foi relatado que níveis séricos mais elevados de AG estão significativamente associados ao desenvolvimento de doenças cardiovasculares, mesmo entre indivíduos sem diabetes ou em aqueles com níveis normais de HbA1c [90]. Essa discordância entre HbA1c e a AG, podem ser explicados pela capacidade da AG refletir variabilidade glicêmica, enquanto HbA1c não reflete a variabilidade glicêmica [84]. Estudo usando monitoramento contínuo de glicose (CGM), relatou que AG, mas não HbA1c, poderia refletir não apenas a glicose média de curto prazo, mas também a variabilidade da glicose plasmática [91]. A importância da variabilidade glicêmica é confirmada em estudos epidemiológicos, onde a glicemia pós-prandial tem um maior risco de causar complicações cardiovasculares e morte em relação à GJ [92, 93].

AG mostrou-se um marcador melhor do que HbA1c para avaliar a presença e gravidade da doença arterial coronariana, e para prever eventos cardíacos adversos maiores em indivíduos com diabetes tipo 2. Valores de AG >20% foram associados a risco relativo de 2,69 [intervalo de confiança (IC) 95% 1,73 – 4,18; p< 0,01)] para doença arterial coronariana, enquanto HbA1c não atingiu significância estatística [94]. Em um estudo prospectivo em pacientes com diabetes tipo 2, foi relatado que a AG é um indicador que prevê a progressão da espessura da íntima-média da carótida e risco de aterosclerose, mas não a HbA1c. Entretanto, a AG estava fortemente correlacionado com HbA1c [95]. Achados semelhantes também foram vistos em outro estudo, onde foi sugerido uso de AG como preditor de desfechos clínicos em longo prazo em pacientes com diabetes tipo 2 e doença arterial coronariana estável [96]. Estudo realizado na Itália demostrou que a adição de AG aos instrumentos tradicionais de controle glicêmico pode melhorar a trajetória clínica de indivíduos com diabetes tipo 2 tratados com apenas terapias

orais, levando a vantagens econômicas e organizacionais para hospitais e Sistemas Nacionais de Saúde [97].

Esses dados sugerem que AG pode ser independente da HbA1c na predição das complicações micro e macrovasculares do diabetes e tem potencial para uso no controle glicêmico como teste adicional aos marcadores tradicionais. No entanto, ainda não existe consenso de metas de controle glicêmico com AG. Além disso, existem poucos estudos em outras populações, inclusive em crianças, gestantes e em indivíduos com estresse induzido por uma enfermidade aguda.

3.4. AG na predição de desfechos adversos em gestante

Durante a gravidez, recomenda-se que mulheres com diabetes prévio ou que desenvolvem DMG mantenham um controle glicêmico rigoroso usando automonitoramento diário da glicemia capilar [98, 99]. Ainda não está bem definido o papel da HbA1c na gestação. A HbA1c está sujeita às alterações hematológicas próprias da gestação e não estão estabelecidos valores de referência para cada trimestre gestacional [100]. A deficiência de ferro sem reposição do mineral pode prolongar a duração das hemácias e pode levar ao aumento da HbA1c [101]. Por sua vez, os valores da HbA1c geralmente caem ao longo da gestação, em razão do aumento da hematopoiese e da diminuição dos níveis de glicose no sangue em jejum, frequentemente observados na gestação [102]. Apesar desses fatores, nas gestações de mulheres com diabetes pré-gestacional, níveis maiores de HbA1c no segundo e no terceiro trimestres estão associados a piores desfechos perinatais [103].

Existem poucos estudos explorando a AG como marcador de controle glicêmico e suas associações com desfechos adversos da gravidez em gestantes. Em estudos que avaliaram mulheres com DMG ou diabetes preexistente, além de demostrarem resultados similares, também reportam que a medida de AG, comparado à HbA1c, foi melhor marcador glicêmico associado à desfechos neonatais adversos [104–108]. Em contrapartida, um estudo demonstrou que a AG tem valor limitado no diagnóstico de DMG e na predição de resultados adversos da gestação [109]. Entretanto, ainda não está definido o papel da AG no DMG e sua relação com os desfechos perinatais.

3.5. AG como marcador glicêmico e na predição de desfechos adversos em COVID-19

Desde o início da pandemia por COVID-19, foi relatado que o grupo de risco para maior gravidade, hospitalização e mortalidade por COVID-19 era formado por pessoas com inúmeras doenças crônicas, entre elas, a mais frequente o diabetes [110–116]. Entretanto, indivíduos com diabetes que tiveram os níveis glicêmicos controlados tiveram melhor prognóstico que pacientes cujos níveis glicêmicos não foram controlados [117, 118]. Em geral, relatam uma associação significativa entre marcadores glicêmicos tradicionais (glicose plasmática e/ou HbA1c) com desfechos adversos da COVID-19 [118–124]; no entanto, poucos estudos mostraram não haver associações [118, 119, 125–127]. Durante a hospitalização, a glicemia é utilizada para monitoramento da glicose, identificação e tratamento de anormalidades glicêmicas; e a HbA1c é recomendada para detectar diabetes não diagnosticado previamente à hospitalização e para orientar as decisões de tratamento do diabetes [79].

A glicose plasmática indica a glicemia sanguínea momentânea e pode ser afetada pelo jejum, ingestão de alimentos e estresse causado por enfermidades agudas, como COVID-19 [79, 128], e também é susceptível às interferências pré-analíticas [6]. Por outro lado, HbA1c não é adequada em condições onde haja tempo de meia vida de hemácias alterado, como transfusão recente, perda de sangue, anemia e DRC [2, 4, 9, 10]. De fato, a anemia frequentemente surge em indivíduos com COVID-19 [129] e os valores de HbA1c nesse grupo pode não refletir com precisão as concentrações de glicose no sangue. Diante desse cenário, é importante considerar opções alternativas de marcadores glicêmicos em pacientes hospitalizados por COVID-19.

Há poucos estudos sobre o papel da AG em COVID-19. Um estudo retrospectivo de 129 adultos com diabetes tipo 2 e com COVID-19 leve avaliou múltiplos marcadores glicêmicos (glicose sérica, HbA1c, AG e razão GA/HbA1c) realizados no momento da admissão e relataram que apenas AG e a razão AG/HbA1c estavam associados de forma independente a um maior risco de progressão de COVID-19 leve para grave. Comparado com indivíduos com AG <20%, indivíduos com AG ≥20% apresentaram maior chance de exacerbação da

COVID-19. Nesse estudo AG foi significativamente correlacionada com HbA1c e GJ [127]. Em outro estudo, o nível de AG foi fortemente associado às mudanças rápidas do estado glicêmico em indivíduos com diabetes e tratamento intensivo, ao passo que a HbA1c muda gradualmente [130]. Também foi relatado que em pacientes no início do tratamento medicamentoso do diabetes ou em terapia intensiva, a AG diminuiu em algumas semanas, enquanto a HbA1c aumentou de forma paradoxal, o que resultou numa discrepância entre as alterações de AG e HbA1c [131].

Esses achados encorajam o uso da AG como marcador glicêmico em pacientes hospitalizados por COVID-19, uma vez que a infecção por SARS-CoV-2 pode levar às mudanças abrutas de glicemia [128]. Entretanto, para seu uso de forma rotineira são necessários mais estudos.

4. LIMITAÇÕES DE AG

Os níveis de AG não são afetados apenas pelos níveis de glicose plasmática, mas também pelo metabolismo da albumina. Hipoalbuminemia está associada ao aumento das taxas de glicação e a albumina compete pela glicação com outras proteínas plasmáticas, tornando-se a mais glicada no meio [132]. Por isso, níveis elevados de AG, independentes da glicemia, podem ser encontrados em pacientes com cirrose hepática ou hipotireoidismo sem tratamento [133, 134]. O hipertireoidismo sem tratamento promove o catabolismo da albumina e resulta em valores baixos de AG, independentes da glicemia [134]. A proteinúria em indivíduos com diabetes com síndrome nefrótica também pode falsamente baixar os valores de AG, enquanto a proteinúria não nefrótica não tem influência significativa nos valores de AG em indivíduos com diabetes e DRC [18, 135]. Devido a isso, AG foi superior a HbA1c na avaliação do controle glicêmico em indivíduos com DRC nos estágios 4 e 5 sem proteinúria maciça, incluindo pacientes em diálise [136, 137].

A AG é influenciado pela idade, índice de massa corporal, massa gorda corporal, tecido adiposo visceral e triglicerídeos [23, 31, 138]. Os mecanismos destas relações permanecem desconhecidos. Entretanto, o índice de massa corporal foi fortemente relacionado de forma não linear à AG, mas linearmente associado à HbA1c e GJ [31].

5. CONCLUSÃO

A AG é um marcador de glicemia de curto prazo e pode refletir melhor variabilidades glicêmicas, quando comparada com HbA1c. AG, por não necessariamente detectar diabetes ou estratificar o risco de desfechos adversos nos mesmos indivíduos identificados pelos testes tradicionais, pode ser usada como um teste complementar na detecção do diabetes e na estratificação de risco de suas complicações. Na prática clínica, a escolha do marcador glicêmico mais adequado para cada situação, depende do conhecimento das características de cada marcador. A seleção apropriada destes marcadores glicêmicos pode ajudar no diagnóstico precoce da diabetes e no planejamento de medidas para prevenir complicações relacionadas à diabetes. No entanto, é necessário um consenso internacional sobre uso clínico da AG, seus pontos de corte e metas de controle glicêmico, para garantir sua inclusão na rotina dos laboratórios clínicos.

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OBJETIVOS

Geral:

Avaliar o uso de albumina glicada (AG) como marcador glicêmico em diferentes contextos clínicos.

Específicos:

1) Realizar revisão sistemática com meta-análise para avaliar a acurácia da AG no diagnóstico de diabetes mellitus na população geral;
2) Avaliar o desempenho da AG no momento da admissão para detectar anormalidades glicêmicas em indivíduos hospitalizados pela doença do coronavírus 2019 (COVID-19);
3) Avaliar a acurácia diagnóstica da AG no diabetes mellitus gestacional (DMG);
4) Avaliar a relação do estado glicêmico definido por teste oral de tolerância à glicose, HbA1c e AG com desfechos gestacionais adversos em gestantes com e sem DMG.

Capítulo 2

Full title: **Glycated albumin in diabetes mellitus: a meta-analysis of diagnostic test accuracy**

Short title: **Glycated Albumin Diagnostic Accuracy for Diabetes**

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ABSTRACT

Background – Guidelines recommend the diagnosis of diabetes should be based on either plasma glucose or glycated hemoglobin (HbA_{1C}) findings. However, lately studies have advocated glycated albumin (GA) as a useful alternative to HbA_{1c}. We conducted a systematic review and meta-analysis to determine the overall diagnostic accuracy of GA for the diagnosis of diabetes.

Methods - We searched for articles of GA diabetes diagnostic accuracy that were published up to August 2021. Studies were selected if reported an oral glucose tolerance test as a reference test, measured GA levels by enzymatic methods, and had data necessary for 2×2 contingency tables. A bivariate model was used to calculate the pooled estimates.

Results - This meta-analysis included nine studies, totaling 10,007 individuals. Of those, 3,106 had diabetes. The studies showed substantial heterogeneity caused by a non-threshold effect and reported different GA optimal cut-offs for diagnosing diabetes. The pooled diagnostic odds ratio (DOR) was 15.93 and the area under the curve (AUC) was 0.844, indicating a good level of overall accuracy for the diagnosis of diabetes. The effect of the GA threshold on diagnostic accuracy was reported at 15.0% and 17.1%. The optimal cut-off for diagnosing diabetes with GA was estimated as 17.1% with a pooled sensitivity of 55.1% (95% CI 36.7% – 72.2%) and specificity of 94.4% (95% CI 85.3% – 97.9%).

Conclusions - GA has good diabetes diagnostic accuracy. A GA threshold of 17.1% may be considered optimal for diagnosing diabetes in previously undiagnosed individuals.

Key words: Diagnosis; Diagnostic accuracy; Diabetes mellitus; Glycated Albumin; Meta-analysis.

List of abbreviations: 2-h PG, 2-h plasma glucose after a 75-g oral glucose tolerance test; ADA, American Diabetes Association; AUC, area under the curve; CI, confidence intervals; DOR, diagnostic odds ratio; FN, false-negative; FP, false-positive; FPG, fasting plasma glucose; GA, Glycated albumin; HbA_{1c}, glycated hemoglobin; HSROC, Hierarchical summary receiver operating characteristic; I2, inconsistency index; LR, likelihood ratios; MeSH, medical subject heading; OGTT, oral glucose tolerance test; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; PROSPERO, Prospective Register of Systematic Reviews; ROC, Receiver Operating Characteristic; STARD, Standard for Reporting Diagnostic Accuracy; SROC, Summary receiver operating characteristic; TN, true-negative; TP, true-positive.

INTRODUCTION

Diabetes is a major health issue that has reached alarming levels: today, nearly half a billion people are living with diabetes worldwide [1]. The condition is chronic and requires continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. The recommendations in the American Diabetes Association (ADA) Standards of Medical Care in Diabetes [2], include screening, diagnostic, and therapeutic actions that are known or believed to favorably affect the health outcomes of patients with diabetes. To date, there is no reference standard definition that captures the phenotypic complexity of diabetes and the risk of its complications. Currently, diabetes may be diagnosed based on either fasting plasma glucose (FPG), 2-h plasma glucose (2-h PG) after a 75-g oral glucose tolerance test (OGTT) or glycated hemoglobin (HbA_{1c}). All tests are equally appropriate and do not necessarily detect diabetes in the same individuals [3, 4].

OGTT is still a standard recommendation with great sensitivity for diabetes diagnosis. Its benefit is the 2-h PG cut point that diagnoses more people with diabetes compared with FPG and HbA_{1c} cut points [3]. However, OGTT measurement lacks reproducibility, it is time-consuming, requires fasting and two blood samples [3, 4].

HbA_{1c}, which is considered the reference for routine monitoring of patients with diabetes, is also a primary diagnostic tool for diabetes. HbA_{1c} has several advantages compared with the FPG and OGTT, including greater convenience (fasting is not required), and greater pre-analytical stability [3]. However, HbA_{1c} is not suitable for conditions with altered erythrocyte turnover, such as hemoglobinopathies, chronic kidney disease and anemia [5]. Those conditions can interfere with the HbA_{1c} measurement and adversely affect the interpretation of HbA_{1c} results [5]. Furthermore, HbA_{1c} ≥6.5% (48 mmol/mol) diagnoses only 30% of the diabetes cases identified collectively using HbA_{1c}, FPG, and/or 2-h PG [6]. Therefore, it is important to consider alternative options in the diagnosis of diabetes.

Glycated albumin (GA), one of the validated tests as an alternative glycemic marker, is produced through the of glucose to albumin in a nonenzymatic reaction [7, 8]. Presently, GA can be measured by enzymatic assays in automated analyzers designed for high throughput. GA is hemoglobin/erythrocyte independent and

reflects the average glucose concentration over the preceding 2–3 weeks, rather than 2–3 months observed for HbA_{1c} [7, 8]. GA, with predictive values alike to HbA_{1c}, it correlates with microvascular and macrovascular outcomes, and even death, especially in people with diabetes [8-12]. Additionally, studies have demonstrated the performance of GA in the diagnosis of diabetes when compared to the performance of HbA_{1c} seems to be similar [13-22]. Therefore, in those studies GA has been proposed as a marker of glycemia that might complement or replace HbA_{1c} under conditions wherein the latter does not reflect glycemic status accurately. However, regardless of the diabetes diagnostic reference standards or the GA thresholds, those studies have been published using varying levels of GA performance [13-22]. Consequently, the use of GA has not been completely endorsed in the diagnosis and screening of diabetes. Thus, to provide more precise summary estimates of clinical performance, we performed a systematic review and meta-analysis of studies that evaluated the performance of GA in the diagnosis of diabetes.

MATERIALS AND METHODS

The protocol of this systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42021265628. In this systematic review and meta-analysis, we followed Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy [23] and conducted the study according to the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement [24].

Search Strategy and Data Sources

With assistance from our Institution's library search specialist, we developed a searching strategy and searched the electronic databases PubMed (MEDLINE) without filter and complemented our search in EMBASE using database filters to remove MEDLINE results. Our search strategy looked for the combination of terms related to "glycated albumin" and "diabetes mellitus" in the title/abstract or across the record and in the medical subject heading (MeSH). This strategy was initially run against the databases in March 2020, and it was updated in August 2021. Details of all search terms are presented in Supplementary Material (Supplemental

Table S1). Duplicate articles were removed from our initial search results, and the remaining articles were assessed for eligibility.

Selection of studies

Studies were selected and included in our final analysis when they met the following criteria: (a) studies that assessed the performance of GA when solely OGTT (reference standard 1) or OGTT and/or HbA_{1c} (reference standard 2) were diabetes diagnostic reference standards; and (b) studies with enrolled individuals older than 18 years. Studies were excluded when: (a) individuals with known diabetes diagnosis or who were receiving anti-diabetic medication were included; (b) GA was measured by a non-enzymatic method; (c) case-control studies; (d) review articles; (e) comments, letters and/or editorials; (f) language other than English, Spanish or Portuguese.

Two review authors (F.C.C. and P.A.C.F.) independently screened titles/abstracts of all reports identified by the literature search and using eligibility criteria coded them as either “potentially include” or “exclude”. Based on the screening results, “potentially include” articles had their full-text assessed for eligibility, using an eligibility assessment form. We reported all excluded studies, with reasons for exclusion, in the PRISMA flow diagram. If multiple publications on a same cohort were found, the latest and most complete publication was considered. Differences in opinion were resolved through discussion or, if required, arbitration by a third review author (J.L.C).

Data extraction and management

Two review authors (F.C.C. and P.A.C.F.) independently extracted data, using a data extraction form, similar to a form previously used by Renz, et al. 2019 [25]. Any disagreements were resolved through discussion, or by consulting a third review author (J.L.C or A.L.P). The following information was extracted from each report: (a) study details (author, publication year, country of origin); (b) study design; (c) sample size; (d) diabetes incidence; (e) participant characteristics [age, gender (male/female), GA, OGTT and HbA_{1c} results]; (f) test methods (details of methodology and equipment description for GA, OGTT and HbA_{1c}); and (g) performance of different cut-offs of GA (sensitivity and specificity, if possible, TP – true-positive cases; FP – false-positive cases; TN – true-negative cases; and FN –

false-negative cases). We also attempted to contact authors for further information when data to construct a 2x2 contingency table was unclear or additional data were required. When data were not available from the authors, the study was excluded.

Quality assessment in included studies

Two review authors (F.C.C. and P.A.C.F.) independently evaluated the risk of bias and applicability of primary studies, using the Quality Assessment of Diagnostic Accuracy Studies tool QUADAS-2. QUADAS-2 consists of four key domains [(i) patient selection; (ii) index test; (iii) reference standard; (iv) flow and timing)], where each is assessed in terms of risk of bias and the first three in terms of concerns regarding applicability. The risk of bias and concerns about applicability were rated as “low,” “high,” or “unclear” [26]. Disagreements were resolved by consensus or by involving a third reviewer (J.L.C. or A.L.P).

Statistical analysis and data synthesis

The standard methods recommended for diagnostic accuracy meta-analysis studies were followed [27]. For each study, 2x2 contingency tables were constructed with data extracted for TP, TN, FP, and FN rates. Summary estimates of sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR−), and diagnostic odds ratio (DOR) with their 95% confidence intervals (CI) were assessed using the bivariate model with random effects approach [28]. Summary receiver operating characteristic (SROC) curves were derived to calculate the area under the curve (AUC) and the Q index. An AUC close to 1 indicates that the diagnostic tests have high discrimination and are meaningful. A high Q index indicates high accuracy of the diagnostic tests. Hierarchical summary ROC curves (HSROC) were used to summarize the GA performance for specific cut-offs if 4 or more studies presented data for the same or rounded cut-off. Fagan’s nomogram was used to present the post-test probabilities for diabetes and pooled sensitivity and specificity were used to present the clinical applicability of the test [28, 30]. A global diabetes prevalence of 9.3% was used as a pre-test probability for diabetes [1]. The heterogeneity among studies was evaluated by visual inspection of forest plots and SROC, Spearman’s correlation coefficient of sensitivity and specificity ($p < 0.05$ indicated significant threshold effect), Cochran’s Q, Chi-square (X2) ($p < 0.10$ indicated significant heterogeneity), and the inconsistency index test (I2). The I2

was defined as: below 30% considered non-important heterogeneity; 30% to 60%, moderate heterogeneity; 60% to 90%, substantial heterogeneity; above 90%, considerable heterogeneity. A high I2 (>50%) and a low p value (<0.05) suggested the presence of heterogeneity caused by the non-threshold effect. The potential publication bias was assessed using Deeks' funnel plot, where $p < 0.1$ indicated statistical significance. Data analysis was performed using Meta-Disc, version 1.4 (Universidad Complutense, Madrid, Spain) and Stata software, Version 12.1 (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 for the present study.

RESULTS

Selection of the Studies

The initial search identified a total of 1,382 records (1,022 from PubMed and 360 from Embase). Of these, 1,358 records were excluded after screening the title/abstract and we fully assessed the remaining 24 records for the eligibility criteria. After full-text assess, 15 articles were excluded (three for different language, 1 used non-enzymatic method for measuring GA, 1 duplicate study population, 9 did not meet the research question or based on eligibility criteria, 1 had insufficient data for 2 × 2 contingency table) (Supplemental Table S2). The remaining 9 articles were eligible for data extraction, of which one article [18] reported two different diagnostic reference standards (OGTT solely and OGTT and/or HbA_{1c}), which was included in the meta-analysis accordingly. Flow diagram is presented in Figure 1.

Characterization of the Studies

The characteristics of each study included in the meta-analysis are shown in Table 1. The included studies were published between 2010 and 2021 and were predominantly performed in Asian countries (four from China; one, Japan; one, Korea; one, Taiwan; one, Brazil; and one, South Africa). Eight studies had cross-sectional design [13, 15, 16 – 21] and one study was community-based cohort study [15]. The number of participants from included studies was 10,007, of those 3,106

(31.0%) were diagnosed with diabetes by the reference method of the individual studies. Six out of 9 included studies assessed the performance of GA in the diagnosis of diabetes by OGTT as the reference test, and had 5,933 participants. Of those, 1,422 (23.9%) were diagnosed with diabetes [13 – 18]. Four studies assessed GA performance using OGTT and/or HbA_{1c} as reference standard and the number of enrolled individuals was 4,316. Of those, 1,770 (41.0%) were diagnosed with diabetes [18 – 21]. The included studies evaluated cut-offs of GA ranging from 13.0% to 17.5%. The Lucica GA-L assay (Asahi Kasei Pharma, Tokyo, Japan) was the most frequently used GA assay (n = 7, 63.6%).

Quality Assessment

The summary of our assessment of the quality of the studies included is reported in Table 2. Five studies had an overall low risk of bias and applicability concerns in all domains of the QUADAS-2 instrument.

One study [20] scored “high” risk of bias in the patient selection, because the flow of participants through the study excluded all individuals with first FPG <7.0 mmol/l and only those who presented a first FPG ≥7.0 mmol/l underwent OGTT and HbA_{1c} with GA. Due to these inclusion criteria, this study scored "high” applicability concerns in the patient selection domain. It also scored “high” risk of bias in the index test domain, because this study used a predefined threshold for optimal cut-off value for GA, obtained by different diagnostic reference standards.

Another study [13] scored “high” risk of bias and applicability concerns in the patient selection because from 908 eligible individuals, 676 were excluded before performing OGTT and GA (633, as normoglycemic with FPG ≤ 5.5 mmol/L, and 43, as newly diagnosed diabetes with FPG ≥ 7.0 mmol/L and/or HbA_{1c} ≥ 6.5%). It also scored “high” risk of bias in the flow and timing domain, because in this study 29 participants who firstly were eliminated with FPG ≥ 7.0 mmol/L criteria were added for Receiver Operating Characteristic (ROC) analyses.

Finally, two studies [16, 21] were not clear in relation to which criterion was used in the patient selection.

Meta-analysis

Overall diagnostic accuracy

For this analysis, we considered GA cut-offs designated as optimal for diagnosing diabetes by the authors of each article [13 – 21]. GA optimal threshold for the diagnosis of diabetes ranged from 14.3% to 17.1%. A total of 10,007 individuals were included in this analysis. Of those, 3,106 were diagnosed with diabetes by the reference method of the individual studies. The pooled DOR was 15.93 (Supplemental Table S3) and the AUC was 0.844 (Supplemental Figure S1). The summary estimate of sensitivity was 0.69 (95% CI: 0.68 – 0.71) and specificity was 0.87 (95% CI: 0.86 – 0.87). There was considerable heterogeneity between studies in terms of sensitivity (Chi-square: 201.77; $p < 0.0001$) and specificity (Chi-square: 552.14; $p < 0.0001$) (Supplemental Table S3). We assumed there was no threshold effect among included studies, since SROC was not shoulder–shaped (Supplemental Figure S1) and Spearman correlation coefficient of sensitivity and specificity was –0.5 ($p = 0.2$). Besides, very low p value and a very high I2 of summary estimates indicated the heterogeneity due to non-threshold effect. The number of studies available has inadequate power to detect the impact of individual quality items as potential sources of heterogeneity. Therefore, we were unable to refine our investigation using meta-regression analyses. However, we performed subgroup analysis, according to the reference test.

Subgroup pooled diagnostic accuracy

As the diagnostic reference standard differed among the studies, we performed subgroup analysis, according to the reference test. The subgroup with OGTT and/or HbA_{1c} as reference standard [18 – 21] had a considerably higher pooled DOR (18.51 vs. 11.91) and diagnostic accuracy (AUC 0.908 vs. 0.772) when compared to the subgroup with OGTT solely [13 – 18]. Pooled estimates of sensitivity, specificity, LR+ and LR– were similar. After re-running the meta-analysis by removing one study at a time, no article explained the persisting high heterogeneity for all summary estimates, regardless of reference standard, and we were unable to elucidate the reasons for this. Detailed accuracy estimates, SROC curves, and heterogeneity test results, according to the subgroup, are provided in the Supplemental Tables S4 and S5, and Supplemental Figures S2 and S3.

Effect of the GA threshold on diagnostic accuracy

The metandi command in Stata software requires a minimum of four studies to compute data [31]. For this reason, to perform this analysis, we used rounded GA cut-offs regardless of the reference test, as a result, only the cut-offs of 15.0% and of 17.1% each gathered at least 4 studies.

GA ≥15.0% for the diagnosis of diabetes

Two studies assessed the performance of GA ≥15.0% to diagnose diabetes by OGTT [15, 18]. The study by Ikezaki, et al. evaluated GA ≥15.2% and Zemlin et al. GA ≥14.9% to diagnose diabetes by OGTT [13, 17]. All cut-offs were rounded to 15.0%, totaling 3,271 individuals. The HSROC curve is presented in Figure 2A. The AUC was 0.72 (Q* = 0.659) (Supplemental Table S6). Forest plots of sensitivity and specificity of the four studies are shown in Figure 3A and the summary of diagnostic accuracy of GA ≥15.0% is presented in Supplemental Table S6. Sensitivity ranged from 62% to 74% and specificity from 62% to 94% (Figure 3A). The pooled sensitivity for these studies was 67.1% (95% CI 60.5% – 73.0%, I2 = 32.1%) and the pooled specificity was 80.9% (95% CI 64.8% – 90.6%, I2 = 98%) (Supplemental Table S6). The pooled LR+ was 3.51 (95% CI 1.74 – 7.05; I2 = 97.0%), LR– was 0.4 (95% CI 0.3 – 0.54; I2 = 72.7%) and DOR was 8.61 (95% CI 3.36 – 22.07; I2=92.69%). Due to the limited number of pooled studies to this meta-analysis, we were unable to perform sensitive analysis to explore the reasons for the considerable heterogeneity among the studies, despite the low p value and high I2 of specificity and DOR indicating heterogeneity due to non-threshold effect. However, the Deeks' funnel plot revealed that there was no significant publication bias (p = 0.19), Supplemental Figure S4A. Considering GA ≥15.0% (with present pooled LR+ and LR–) as diabetes diagnostic criterion and inferring in global population with pre-test probability of 9.3% for diabetes [1], after a positive test (GA ≥15.0%) the post-test probability for diabetes would increase to 26%, while a negative test (GA <15.0%) would decrease the post-test probability for diabetes to 4% (Figure 4A).

Apart from the above cited studies [13, 15, 17, 18], we also performed a meta-analysis to assess diabetes diagnostic accuracy of GA ≥15.0%, including one study that evaluated GA ≥15.15% using OGTT and/or HbA_{1c} as reference standard [21].

Pooling these studies together, the total of individuals was 5,206 (Figure 2B), the combined sensitivity was 74% (95% CI 60% – 84%; I2 = 93.5%) and combined specificity was 81% (95% CI 68% – 89%; I2 = 98.1%). Those results are similar to the one found without the study by Li, et al. [21], but worsen the heterogeneity between studies. Furthermore, the Deeks' funnel plot revealed that there was significant potential publication bias (p = 0.04), Supplemental Figure S4B. Therefore, the results from the primary meta-analysis for this cut-off (GA ≥15.0%) were considered.

GA ≥17.1% for the diagnosis of diabetes

Three studies reported the performance of GA ≥17.1% for diagnose diabetes by OGTT [14, 15, 18]. One study evaluated the threshold of GA ≥17.1% to diagnose diabetes using OGTT and/or HbA_{1c} as reference standard [17]. All four studies totalled 5,059 individuals. The HSROC curve is shown in Figure 2C. The AUC was 0.85 (95% CI 0.82 – 0.88; Q* = 0.7775) (Supplemental Table S6). Forest plots of sensitivity and specificity of the four studies are shown in Figure 3C and the summary of diagnostic accuracy of GA ≥17.1% is presented in Supplemental Table S6. Sensitivity ranged from 29% to 77% and specificity from 77% to 98% (Figure 3C). The pooled sensitivity was 55.1% (95% CI 36.7% – 72.2%, I2 = 97.3%) and specificity was 94.4% (95% CI 85.3% – 97.9%, I2 = 99.2%) (Supplemental Table S6). The pooled LR+ was 9.78 (95% CI 4.29 – 22.34; I2 = 97.5%), LR– was 0.47 (95% CI 0.33 – 0.69; I2 = 97.3%) and DOR was 20.56 (95% CI 9.01 – 46.94; I2 = 93.1%). Again, we were unable to perform sensitive analysis to explore the reasons for the considerable heterogeneity between studies in pooled indexes. The Deeks' funnel plot showed no significant publication bias (p = 0.76), Supplemental Figure S4C. Applying the Fagan's nomogram with pre-test probability of 9.3% for diabetes [1], the post-test probability for diabetes would increase to 50% after a positive test (GA ≥17.1%), while a negative test (GA <17.1%) would decrease the post-test probability for diabetes to 5% (Figure 4B).

DISCUSSION

Summary of main results

Our results showed that when examining GA at designated as optimal cut-offs (by the authors of each primary study) for the diagnosis of diabetes, pooled sensitivity and specificity were 0.69 and 0.87, respectively. The diagnostic test exhibited high discrimination (AUC = 0.8442 with a Q* value of 0.7757) and good determination effect (DOR = 15.93). When we splitted the studies into subgroups according to reference standard, the subgroup with OGTT and/or HbA_{1c} had a considerably higher pooled DOR and AUC than the subgroup with OGTT solely. Pooled sensitivity, specificity, LR+ and LR– of the two subgroups were similar to each other and were almost equal to the overall pooled estimates of primary analysis.

We presented the effect of the GA threshold at rounded values of 15.0% and 17.1%. For a rounded cut-off of 15.0%, the pooled sensitivity and specificity was 0.671 and 0.809, respectively. This accuracy implies 0.329 of false-negative and 0.191 of false-positive. The AUC and DOR suggested good determination effect and acceptable diagnostic accuracy. The pooled LR+ and LR– indicated that the pre-test to post-test probabilities would generate a minimal change, though significant. In comparison to the cut-off of 15.0%, the threshold of 17.1% showed lower pooled sensitivity (0.551) and false-positive (0.056), but greater pooled specificity (0.944) and false-negative (0.449). The AUC was 0.85 and DOR was 20.56, suggesting good determination effect and great diagnostic accuracy. The pooled LR+ indicated that the post-test probability for diabetes would moderate increase after a positive test, while the pooled LR– indicated that the pre-test to post-test probabilities, though significant, would generate a minimal change after a negative test.

Our results compared with other reports

As far as we know, this is the first systematic review with meta-analyses to evaluate the accuracy of the GA at the cut-offs of 15.0% and of 17.1% in the diagnosis of diabetes. In a recent systematic review and meta-analyses [32] that aimed to summarize the available data on GA measurements for the diagnosis of diabetes authors reported the accuracy of the GA at the cut-offs of 14.0%. The summary estimate of sensitivity was 0.766, specificity was 0.687, an AUC of 0.80 and DOR of 7.176. However, meta-analyzed data included sample from select populations, such as kidney transplant recipients [33] and youths 10 to 18 years-old with cystic fibrosis [34].

The results of GA diagnostic accuracy in our meta-analysis showed similar results to another meta-analysis conducted to evaluate the accuracy of the HbA_{1c} in the diagnosis of diabetes, where both GA and HbA_{1c} at optimal thresholds presented higher values of pooled specificity than sensitivity [35 – 37]. Summary estimates of GA ≥17.1% compared with other reports summary estimates of HbA_{1c} ≥6.5% for the diagnosis of diabetes are presented in Supplemental Table S7. Our findings in terms of pooled sensitivity (0.551) for the GA ≥17.1% are slightly higher than those reported elsewhere in meta-analysis that assessed the diagnostic value of HbA_{1c} ≥6.5% for diabetes by Xu et al. (0.518) [35] and Kaur et al (0.50) [36], it was even higher when compared with pooled sensitivity reported by NCD-RisC group (0.305) [37]. So, GA ≥17.1% had lesser false-negative cases compared to HbA_{1c} ≥6.5%. However, our pooled sensitivity is lower than the one reported by Hoyer et al. (0.551 vs.0.684) [38]. On the contrary, our finding of pooled specificity for GA 17.1% (0.944) is lower than that reported in HbA_{1c} 6.5% by Xu et al. (0.956) [35], Kaur et al, (0.973) [36], NCD-RisC group (0.997) [37] and by Hoyer et al. (0.959) [38]. Thus, GA ≥17.1% had higher false-positive cases than HbA_{1c} ≥6.5%. Further, GA 17.1% presented lower diagnostic accuracy (AUC = 0.85 vs. 0.93), and determination effect (DOR = 20.7 vs. 40.6) than those for HbA_{1c} 6.5% reported by Xu et al. [37]. The pooled LR+ was also lower (9.78) than that reported by Xu et al. (19.0) [35] and by Kaur et al. (18.32) [36], which indicates that HbA_{1c} of 6.5% presents greater post-test probability for diabetes than GA of 17.1% after a positive test result. The GA 17.1% had similar pooled LR– (0.47 vs. 0.48) as for HbA_{1c} 6.5% estimated by Xu et al. [35], and slightly lower than those estimated by Kaur et al

(0.51) [36]. The pooled LR– indicates both GA and HbA_{1c} would generate a minimal change of pre-test to post-test probabilities after a negative test result.

Applicability of findings to the review question

To make sense of the results of the meta-analysis and its applicability in clinical practice, we explored pooled sensitivity and specificity, and the post-test probabilities for diabetes applying the Fagan's nomogram. A global diabetes prevalence of 9.3% was used as pre-test probability for diabetes [1] with pooled LR+ and LR– for GA cut-offs 15.0% and 17.1%. After a test, the post-test probability for diabetes would increase to 26% for GA ≥15.0% and 50% for GA ≥17.1%. The post-test probability would decrease to 4% for GA <15.0% and 5% for GA <17.1%. Using GA ≥15.0% to diagnose diabetes with the pooled sensitivity of 0.671 and specificity of 0.809, for every 1,000 individuals tested, 62 cases of diabetes would be detected, 31 cases would be missed, and there would be 173 false diabetes diagnoses. For GA ≥17.1% as diabetes diagnostic criterion with the pooled sensitivity of 0.551 and specificity of 0.944, we estimate for every 1,000 individuals tested 51 cases of diabetes would be detected, 42 cases would be missed, and there would be 51 false diabetes diagnoses. Even though GA ≥17.1% presents lower diagnostic accuracy with higher false-positive results than HbA_{1c} ≥6.5%, its higher sensitivity than HbA_{1c} ≥6.5% [35 – 37] may have important implications from both clinical and healthcare policy perspectives. The alarming increase in the prevalence of diabetes worldwide warrants tests with greater sensitivity without meaningful loss of specificity for the early identification of the disease [1]. Thus, based on our findings the GA thresholds of 17.1% for screening purposes may be considered, once an early preventive intervention for people at high risk and treatment for newly diagnosed can help in reducing the incidence of diabetes complications, including cardiovascular morbidity and mortality [39].

Fang *et al.* analyzed the data from a multiethnic community-based cohort (n = 4785), and suggested GA had excellent diagnostic accuracy, with the AUC ranging from 0.824 to 0.951. GA cut-offs of 16.5% and 17.8% were, respectively, equivalent to an FPG of 126 mg/dL (97th percentile) and HbA_{1c} of 6.5% (98th percentile) and had low to moderate sensitivity (0.273 to 0.707) but high specificity (0.980 to 0.992) for detecting undiagnosed diabetes. However, the reference definitions adopted in

this study were without OGTT [FPG (≥126 mg/dL), HbA_{1c} (≥6.5%), either FPG or HbA_{1c} increased, or both FPG and HbA_{1c}] [40]. Another study by Araki *et al.* in Japanese people reported a very efficient strategy to improve the metabolic control status of a general population using GA measurement as a screening tool for diabetes [41]. In the study, traditional glycemic tests were dispensed, and GA values were used to define the glycemic status and clinical practice in approximately 3 million people [41]. Based on Araki *et al.* definition, our finding of optimal threshold of GA as 17.1% for the screening for diabetes in previously undiagnosed population lies within the range of prediabetes (16.5 – 18.3%) [41]; and that is close to the "optimal" cut-offs estimated by Fang *et al.* and by several included studies [14, 15, 18, 20, 40]. It is noteworthy to mention that the risk of all-cause and cardiovascular mortality starts in the prediabetes stage even before clinical diabetes sets in and may also lead to significant morbidities as well [12, 42]. This behavior is essentially explained by the fact that there is no reference standard definition that captures the phenotypic complexity of diabetes and the risk of its microvascular and macrovascular complications. Consequently, all tests are equally appropriate to diagnose diabetes, although OGTT normally ranks high with great sensitivity for diabetes diagnosis [3, 4, 43, 44].

Although the GA is also relatively easy to use (fasting not required and measurement stability) and presents higher sensitivity for diabetes than the HbA_{1c}, when GA is used, traditional glycemic tests should ideally also be measured. Because the number of false-negative for GA persisted considerably high, therefore, using GA alone in health surveys might miss some previously undiagnosed people who would be considered as having diabetes using a glucose-based test and/or HbA_{1c}, and under these circumstances, could benefit from lifestyle and treatment interventions. This does not diminish the importance of GA in the diagnosis of diabetes, because adding GA to traditional glycemic test instruments could improve the clinical pathway of individuals with diabetes and healthcare systems.

Strengths and weaknesses of the review

A major strength of this review is that we conducted an extensive and systematic literature search without filter, which ensured we included all studies that

met the inclusion criteria, and, in the case of missing data, we attempted to contact the authors to improve the data extraction. Three diagnostic test accuracy studies [40 45 – 47] that assessed the performance of GA without OGTT in reference standard did not meet eligibility criteria but after re-running the meta-analysis including those studies, the summary estimates were not significantly different from the primary meta-analysis (results not shown).

This study presents certain limitations. Although the findings were generally similar in the studies included, the meta-analysis revealed that there was considerable heterogeneity among them. Even after excluding two studies [13, 20] that we judged to be at high risk of bias and applicability concerns, and omitting the other studies one at a time, the analysis persisted very similar to our initial results. The attempt of performing a subgroup analysis according to the diagnostic reference standard or using rounded or same diagnostic cut-off values of GA was also not able to decrease heterogeneity. Our analysis suggested that the presence of heterogeneity was caused by a non-threshold effect. The small number of studies available hampered other types of subgroup analyses and a full explanation for the significant amount of heterogeneity found among studies. The minimum number of studies required for regression analysis is ten, otherwise we would have inadequate power to detect the potential sources of heterogeneity [48 – 50].

It should also be noted that our meta-analysis results are based on test accuracy data reported by primary studies conducted in settings with a disease prevalence exceeding that in most national/local prevalence [1]. Another limitation found is that most studies included in the present meta-analysis were undertaken in Asian countries, most notably in China. This may limit the generalization of our findings and indicates a need for further evaluations of test performance in different ethnicities.

We could not assess all objectives planned for this review due to limitations in data availability, highlighting an information gap. Studies were not consistent in using the same thresholds for GA in the diagnosis of diabetes. As a result, we were unable to fully assess the effect of different GA thresholds on diagnostic accuracy. We created subgroups with a rounded cut-off value and/or neglected the diagnostic reference standard, which enabled us to evaluate the effect at two GA thresholds.

Therefore, our findings should be interpreted with caution. And, not to perpetuate missing data, it is extremely important that future studies are designed and reported according to the Standard for Reporting Diagnostic Accuracy (STARD) statement [51]. We also suggest reporting data of sensitivity and specificity of multiple GA cut-off points (e.g., 14.0%; 14.5%; 15.0%; 15.5%; 16.0%; 16.5%; 16.6%; 16.8%; 17.0%;17.1%; 17.2%...).

Conclusions

GA performance in the diagnosis of diabetes is similar to HbA_{1c}. Both GA and HbA_{1c} result in few false-positive diabetes cases, but high number of false-negatives diabetes cases. The GA threshold of 17.1% may be considered optimal for diagnosing diabetes in previously undiagnosed individuals and would be more sensitive than HbA_{1c} ≥6.5%, with no meaningful loss of specificity. Since the number of false-negatives for GA 17.1% persisted considerably high, a negative result should ideally go for further investigation through a different test for diagnosis confirmation. Thus, GA may be used more of an additional test than an alternative to traditional glycemic tests, including HbA_{1c}. The use of GA in surveillance requires further consideration of how it predicts and helps prevent diabetes complications and it is beyond the scope of this review. Furthermore, careful consideration about standardization of GA assays would be necessary, as has been done for HbA_{1c}, to yield highly consistent GA results and increase precision.

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Author Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

F.C.C., J.L.C. conceptualization; F.C.C., J.L.C., study design; F.C.C., literature search; F.C.C., L.G.S., literature review; F.C.C., P.A.C.F., data extraction and compilation; F.C.C., A.L.P, statistical analysis; F.C.C., J.L.C., manuscript writing and revision; All authors were involved in reviewing, discussing data, and commented on the manuscript.

Competing interests

Authors state no conflict of interest.

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Table 1. Characteristics of selected studies.

Author (Reference)	**Study location**	**Study design**	**Sample size (n)**	**Age (Years)**	**GA (%)**	**Reference Standard**	**Incidence of diabetes (%)**	**GA cut-off (S & E) †**	**GA method**
Ma, et al. 2010	China	Cross-sectional	1971	53.1 ± 14.6	17.86 ± 4.5	OGTT	38.30	**17.1% (76.82% & 76.89%)**	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan) on Glamour 2000 autoanalyzer. GA
Hwang, et al. 2014	Korea	Cross-sectional	852	52.5 ± 10.3	14.2 ± 5.6	OGTT + HbA_{1c}	37.08	**14.3% (66.4% & 88.3%)**	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan) on Hitachi 7699 Pmodule autoanalyzer (Hitachi Instruments Service)
Ikezaki, et al. 2015	Japan	Cross-sectional	176	Men 60 (53, 63) Women 58 (51, 63)	Men 13.8 (13.0, 14.9) Women 14.4 (13.7, 15.3)	OGTT	16.5	**15.2% (62.1% & 61.9%)**; 16.5% (34.5% & 87.1%)	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan)
Wu, et al. 2016	Taiwan	community-based cohort	1559	50.4 ± 12.6	14.0 ± 2.6	OGTT	8.5	14.0% (83.33% & 63.28%) ‡ 14.5% (78.03% & 76.94%) ‡ 15.0% (74.0% & 85.0%) 15.5% (68.94% & 90.96%) ‡ 16.0% (62.12% & 94.81%) ‡ 16.3% (56.06% & 96.71%) ‡ 16.5% (55.3% & 96.92%) ‡ **17.0% (47.73% & 98.18%)** ‡ 17.1% (46.21% & 98.32%) ‡ 17.5% (41.67% & 98.95%) ‡	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan) on Beckman Coulter AU2700 Chemistry Analyzer (Beckman Coulter Systems Co., Nyon, Switzerland)

He, et al. 2017	China	Cross-sectional	1287	55 (47–62)		OGTT + HbA_{1c}	77.08	**17.1% (63.41% & 95.93%)**	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma) on 7600 chemistry analyzer (Hitachi).
Su, et al. 2018	China	Cross-sectional	691	50.5 ± 13.3	16.2 ± 3.1	OGTT	48.5	**16.3% (67.5% & 83.4%)**	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan) on Hitachi 7600–120 (Hitachi, Tokyo, Japan)
Chume, et al. 2019	Brazil	Cross-sectional	242	53.4 ± 13.4	14.9 ± 2.2	OGTT OGTT + HbA_{1c}	By OGTT 31.8 By OGTT + HbA_{1c} 35.5	13.0% (93.5% & 15.2%); 14.0% (84.4% & 44.2%); 14.5% (70.1% & 57.6%) ‡ 14.8% (64.9% & 65.5%); 15.0% (62.3% & 69.7%); 15.5% (48.1% & 77.6%); 16.0% (42.9% & 84.8%); 16.3% (42.9% & 87.9%) ‡ **16.6% (36.4% & 90.3%);** 16.8% (31.2% & 93.3%); 17.0% (29.9% & 93.9%); 17.1% (28.6% & 93.9%) ‡ 17.5% (20.8% & 96.4%); 14.7% (64.0% & 64.1%) § 16.6% (33.7% & 90.4%) ‡§	Enzymatic method (GlycoGap, Diazyme Laboratories, Poway, CA) in Cobas c702 (Roche Diagnostics, Germany)
Zemlin, et al. 2019	South Africa	Cross-sectional	1294	47.8 ± 15.5	13.3 ± 2.7	OGTT	7.3%	**14.9% (64.8% & 93.5%)**	Enzymatic method (quantILab Glycated Albumin assay, Werfen™, Italy) in Roche cobas 6000 analyzer (Roche Diagnostics, Germany)

Li, et al. 2021	China	Cross-sectional	1935	NGT 28.11 ± 5.44 Pre-DM 37.15 ± 12.81 DM 47.63 ± 13.44	NGT 12.36 ± 0.81 Pre-DM 13.69 ± 1.45 DM 18.35 ± 5.00	OGTT + HbA_{1c}	19.431%	**15.15% (90.7% & 78.9%)**	Enzymatic method (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan) on Cs400B (Dirui Industrial Co., Ltd., Changchun, China)

Data are expressed as mean±SD or median (interquartile range); † GA designated optimal threshold in diagnosis of diabetes in bold; ‡ Data supplied by the author after contact; § OGTT and/or HbA_{1c} are reference; GA, glycated albumin; OGTT, oral glucose tolerance test; HbA_{1c}, glycated hemoglobin; S & E, sensitivity and specificity; DM, Diabetes mellitus; NGT, Normal glucose tolerance.

Table 2: Quality assessment using QUADAS-2 criteria.

Study		Risk of Bias				Applicability concerns		
		Patient Selection	Index Text	Reference Standard	Flow and Timing	Patient Selection	Index Text	Reference Standard
1	Ma, et al. 2010	☺	☺	☺	☺	☺	☺	☺
2	Hwang, et al. 2014	☺	☺	☺	☺	☺	☺	☺
3	Ikezaki, et al. 2015	☹	☺	☺	☹	☹	☺	☺
4	Wu, et al. 2016	☺	☺	☺	☺	☺	☺	☺
5	He, et al. 2017	☹	☹	☺	☺	☹	☺	☺
6	Su, et al. 2018	?	☺	☺	☺	☺	☺	☺
7	Chume, et al. 2019	☺	☺	☺	☺	☺	☺	☺
8	Zemlin et al. 2019	☺	☺	☺	☺	☺	☺	☺
9	Li, et al. 2021	?	☺	☺	☺	☺	☺	☺

☺ low; ☹ high; ? unclear.

Figure Legends

Figure 1: Flowchart of the article selection process.

Figure 2: Hierarchical summary receiver operating characteristic curves.

(A) GA ≥15.0% to diagnose diabetes by OGTT; (B) GA ≥15.0% to diagnose diabetes regardless of the reference standard: OGTT solely or OGTT and/or HbA_{1c}; (C) GA ≥17.1% to diagnose diabetes regardless of the reference standard: OGTT solely or OGTT and/or HbA_{1c}. GA, glycated albumin; OGTT, oral glucose tolerance test; HbA_{1c}, glycated hemoglobin.

Figure 3: Forest plots of estimates of sensitivity and specificity in each study.

(A) GA ≥15.0% to diagnose diabetes by OGTT; (B) GA ≥15.0% to diagnose diabetes regardless of the reference standard: OGTT solely or OGTT and/or HbA_{1c}; (C) GA ≥17.1% to diagnose diabetes regardless of the reference standard: OGTT solely or OGTT and/or HbA_{1c}. TP, true positive; FP, false positive; FN, false negative; TN, true negative. GA, glycated albumin; OGTT, oral glucose tolerance test; HbA_{1c}, glycated hemoglobin.

Figure 4: Fagan's nomogram for GA, showing post-test probabilities for diabetes.

GA ≥15.0% and (B) GA ≥17.1%. GA, glycated albumin.

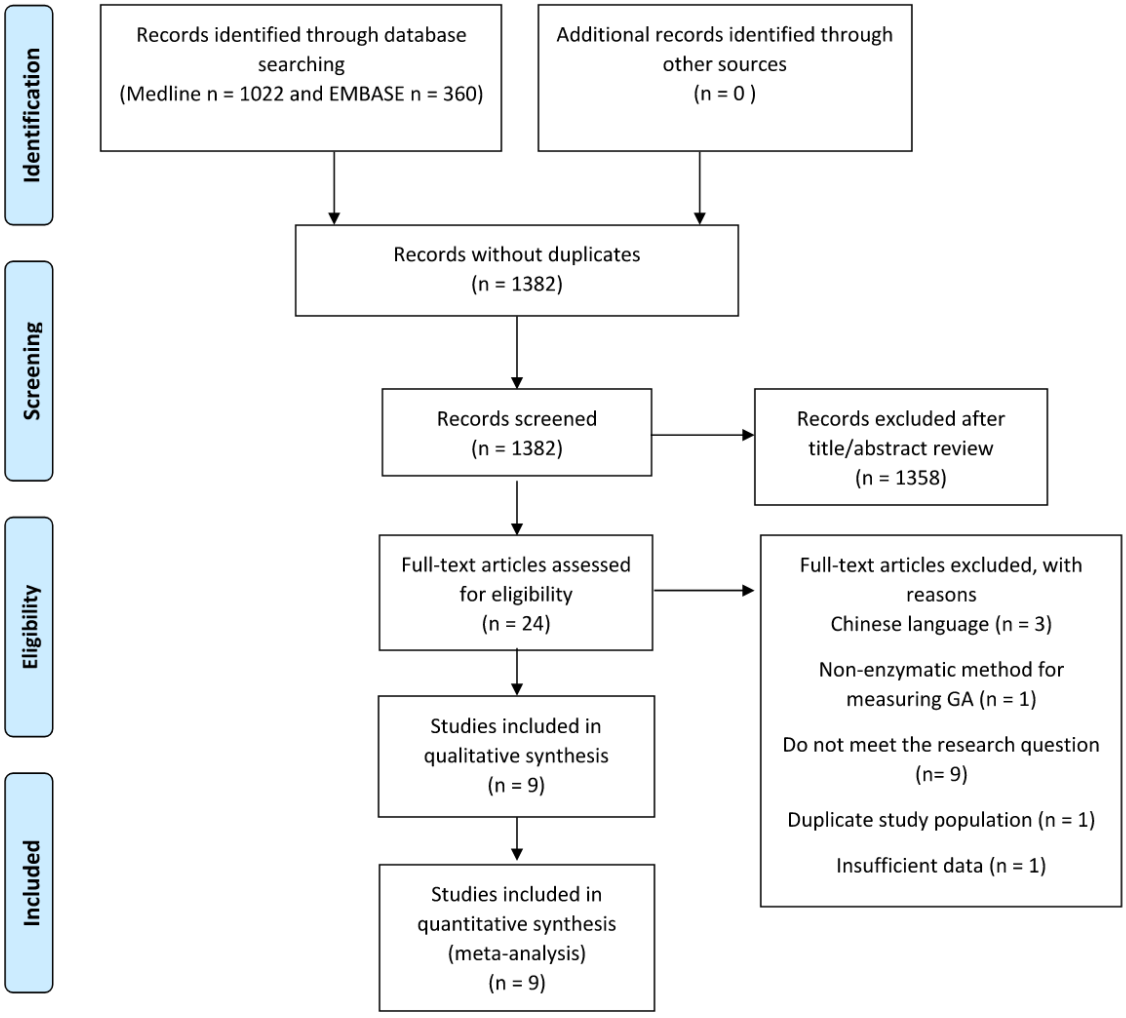

Identification
Screening
Eligibility
Included
Records identified through database searching
(Medline n = 1022 and EMBASE n = 360)
Additional records identified through other sources
(n = 0)
Records without duplicates
(n = 1382)
Records screened
(n = 1382)
Records excluded after title/abstract review
(n = 1358)
Full-text articles assessed for eligibility
(n = 24)
Full-text articles excluded, with reasons
Chinese language (n = 3)
Non-enzymatic method for measuring GA (n = 1)
Do not meet the research question (n= 9)
Duplicate study population (n = 1)
Insufficient data (n = 1)
Studies included in qualitative synthesis
(n = 9)
Studies included in quantitative synthesis (meta-analysis)
(n = 9)

Figure 1

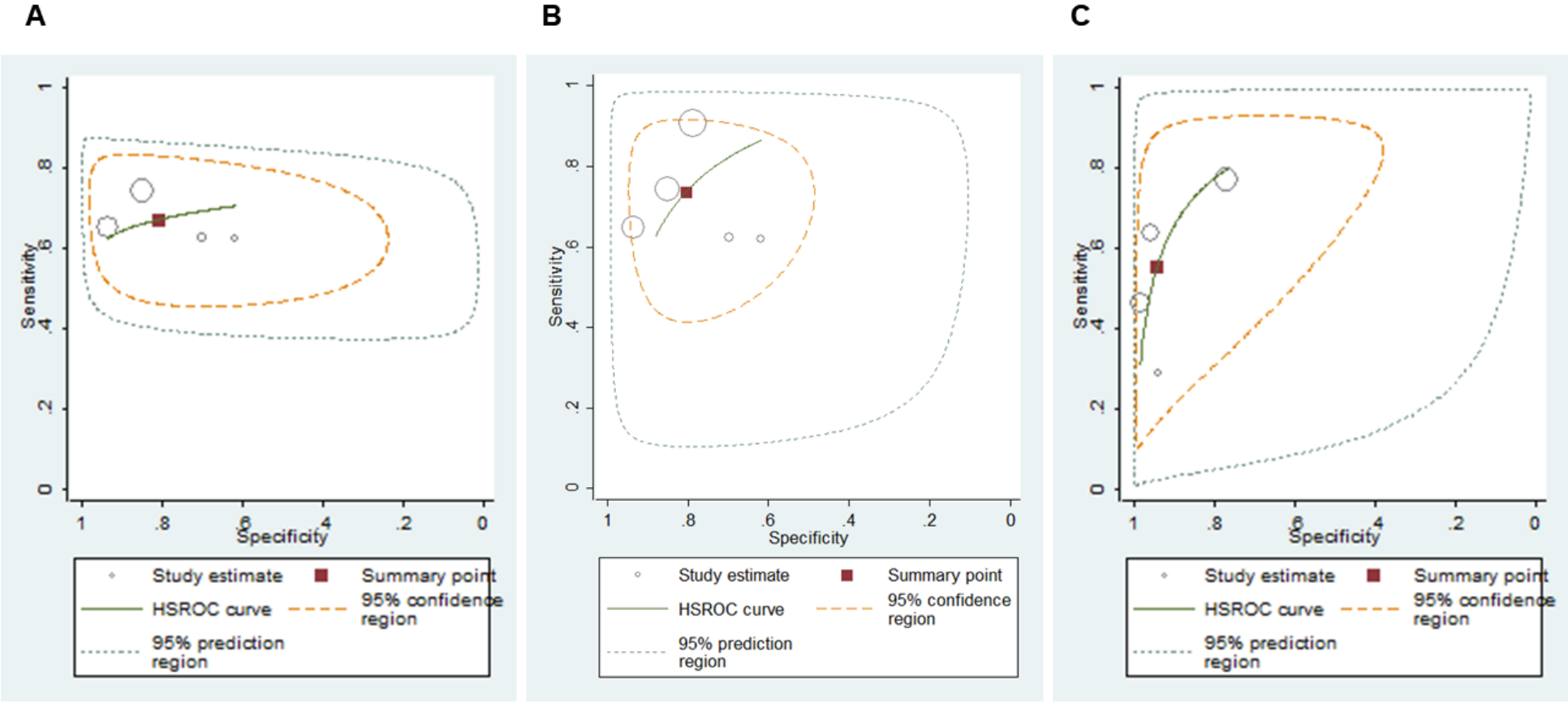
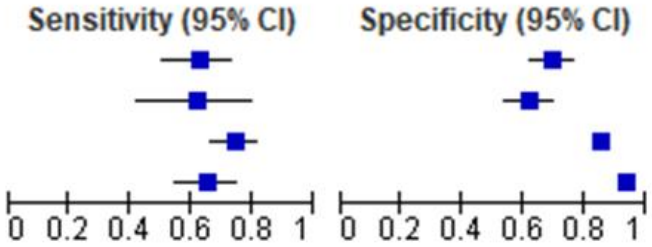

A
B
C
Sensitivity
Specificity
1
.8
.6
.4
.2
0
Study estimate
Summary point
HSROC curve
95% confidence region
95% prediction region

Figure 2

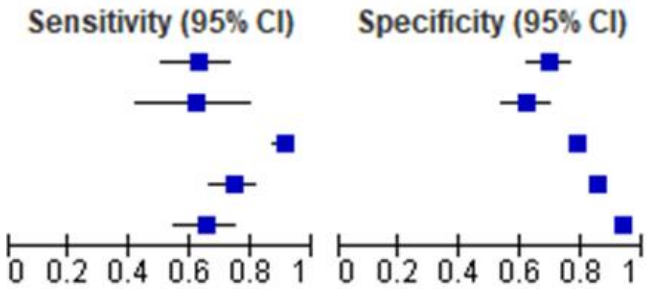
A

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Chume, et al. 2019	48	50	29	115	0.62 [0.51, 0.73]	0.70 [0.62, 0.77]
Ikezaki, et al. 2015	18	56	11	91	0.62 [0.42, 0.79]	0.62 [0.54, 0.70]
Wu, et al. 2016	98	214	34	1213	0.74 [0.66, 0.81]	0.85 [0.83, 0.87]
Zemlin, et al. 2019	61	78	33	1122	0.65 [0.54, 0.74]	0.94 [0.92, 0.95]



B

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Chume, et al. 2019	48	50	29	115	0.62 [0.51, 0.73]	0.70 [0.62, 0.77]
Ikezaki, et al. 2015	18	56	11	91	0.62 [0.42, 0.79]	0.62 [0.54, 0.70]
Li, et al. 2021	341	329	35	1230	0.91 [0.87, 0.93]	0.79 [0.77, 0.81]
Wu, et al. 2016	98	214	34	1213	0.74 [0.66, 0.81]	0.85 [0.83, 0.87]
Zemlin, et al. 2019	61	78	33	1122	0.65 [0.54, 0.74]	0.94 [0.92, 0.95]



C

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Chume, et al. 2019	22	10	55	155	0.29 [0.19, 0.40]	0.94 [0.89, 0.97]
He, et al. 2017	629	12	363	283	0.63 [0.60, 0.66]	0.96 [0.93, 0.98]
Ma, et al. 2010	580	281	175	935	0.77 [0.74, 0.80]	0.77 [0.74, 0.79]
Wu, et al. 2016	61	24	71	1403	0.46 [0.38, 0.55]	0.98 [0.98, 0.99]

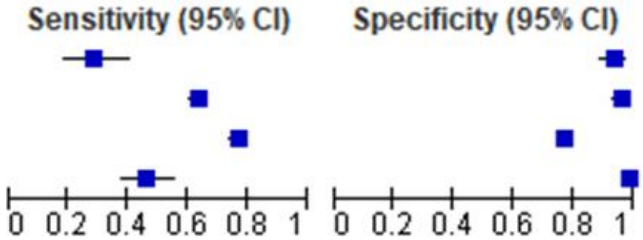


Figure 3

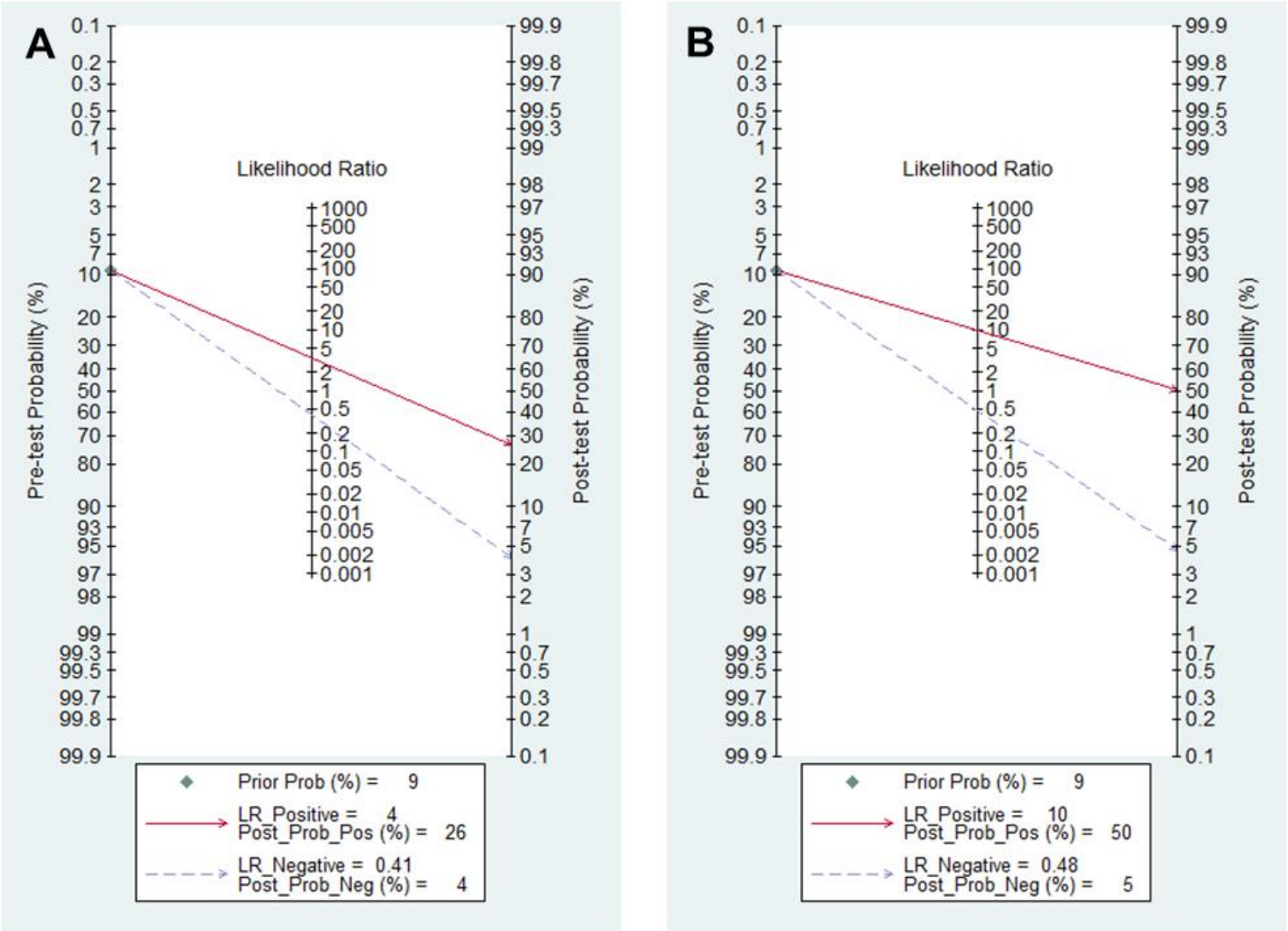

A
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 9
LR_Positive = 4
Post_Prob_Pos (%) = 26
LR_Negative = 0.41
Post_Prob_Neg (%) = 4
B
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 9
LR_Positive = 10
Post_Prob_Pos (%) = 50
LR_Negative = 0.48
Post_Prob_Neg (%) = 5

Figure 4

Supplementary Materials

(Glycated albumin in diabetes mellitus: a meta-analysis of diagnostic test accuracy)

Supplemental Table S1. Search details of all terms

Databases†	Keywords for Electronic Searches	Translations of Keywords with Search Query	Search results
PubMed	Glycated albumin Diabetes Mellitus	glycosylated serum albumin[mh] OR glycosylated serum albumin[tw] OR glycosyl-albumin[tw] OR glycated albumin[tw] OR glycoalbumin[tw] OR glucosylated albumin[tw] AND Diabetes Mellitus, Type 2[mh] OR Diabetes Mellitus, Type 2[tw] OR Ketosis-Resistant Diabetes Mellitus[tw] OR Non-Insulin-Dependent[tw] OR Diabetes Mellitus[tw] OR Stable Diabetes Mellitus[tw] OR Diabetes Mellitus, Type II[tw] OR NIDDM[tw] OR Maturity-Onset Diabetes Mellitus[tw] OR Maturity Onset Diabetes Mellitus[tw] OR MODY[tw] OR Slow-Onset Diabetes Mellitus[tw] OR Type 2 Diabetes Mellitus[tw] OR Noninsulin-Dependent Diabetes Mellitus[tw] OR Noninsulin Dependent[tw] OR Diabetes Mellitus[tw] OR Maturity-Onset Diabetes[tw] OR Maturity Onset Diabetes[tw] OR Type 2 Diabetes[tw] OR Adult-Onset Diabetes Mellitus[tw]	1022
EMBASE	Glycated albumin Diabetes Mellitus	'glycosylated albumin'/exp OR "glycosylated albumin":ti,ab,kw OR "glycosylated serum albumin":ti,ab,kw OR "glycosyl-albumin":ti,ab,kw OR "glycated albumin":ti,ab,kw OR "glycoalbumin":ti,ab,kw OR "glucosylated albumin":ti,ab,kw AND 'non insulin dependent diabetes mellitus'/exp OR "Diabetes Mellitus, Type 2":ti,ab,kw OR "Ketosis-Resistant Diabetes Mellitus":ti,ab,kw OR "Non-Insulin-Dependent":ti,ab,kw OR "Diabetes Mellitus":ti,ab,kw OR "Stable Diabetes Mellitus":ti,ab,kw OR "Diabetes Mellitus, Type II":ti,ab,kw OR "NIDDM":ti,ab,kw OR "Maturity-Onset Diabetes Mellitus":ti,ab,kw OR "Maturity Onset Diabetes Mellitus":ti,ab,kw OR "MODY":ti,ab,kw OR "Slow-Onset Diabetes Mellitus":ti,ab,kw OR "Type 2 Diabetes Mellitus":ti,ab,kw OR "Noninsulin-Dependent Diabetes Mellitus":ti,ab,kw OR "Noninsulin Dependent":ti,ab,kw OR "Diabetes Mellitus":ti,ab,kw OR "Maturity-Onset Diabetes":ti,ab,kw OR "Maturity Onset Diabetes":ti,ab,kw OR "Type 2 Diabetes":ti,ab,kw OR "Adult-Onset Diabetes Mellitus":ti,ab,kw	360

†Searches updated on August 11, 2021

Supplemental Table S2. List of studies excluded at full-text screening stage, with brief reasons.

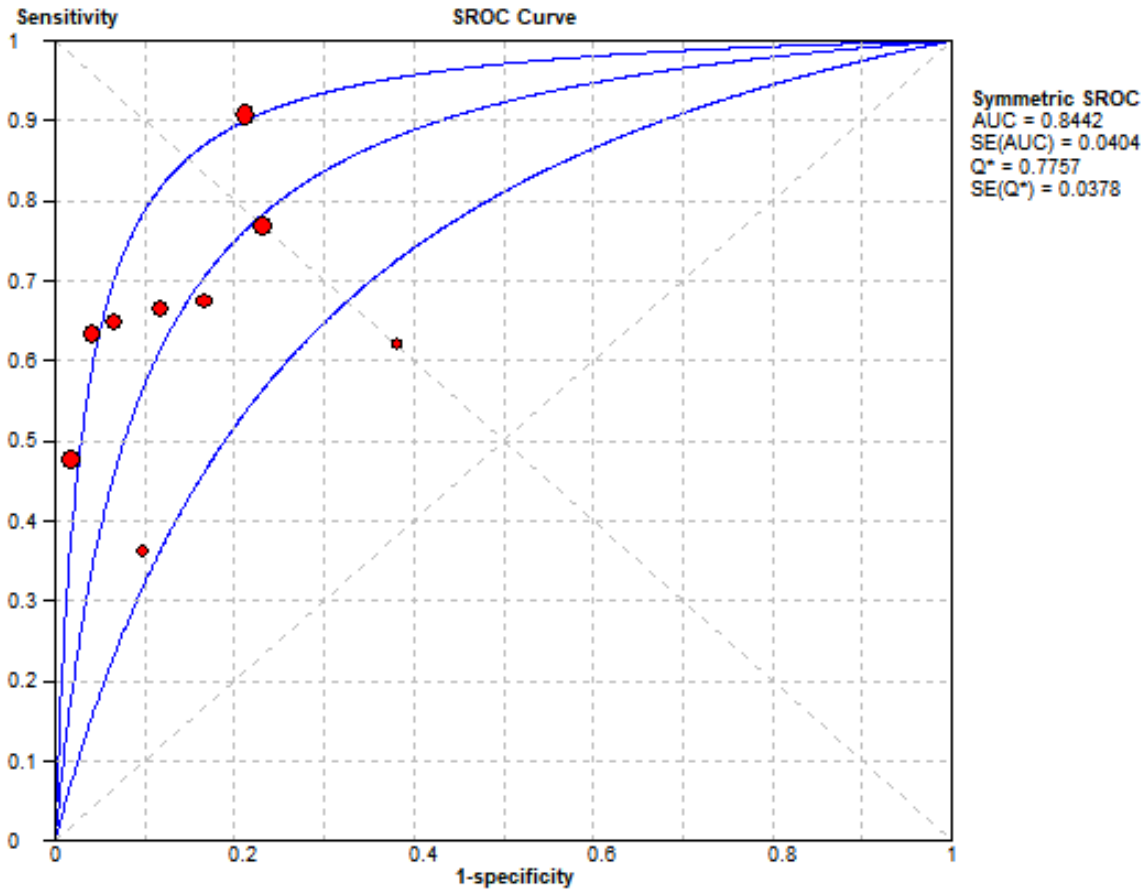
Reason	Study excluded	
Different language	1	Li Q, Pan JM, Ma XJ, Bao YQ, Tang JL, Yuan QY, Lu HJ, Jia WP. [Combined utility of hemoglobin A1c and glycated albumin in diabetic screening]. Zhonghua Yi Xue Za Zhi. 2011 Jul 12;91(26):1813-6. Chinese.
	2	Zhang T, He H, Yang HL, Huang HJ, Zhang MF, An ZM, Li SQ. [Study of glycated albumin cut-off point in diabetes mellitus and impaired glucose regulation]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2014 Mar;45(2):274-7, 298. Chinese.
	3	Su H. Efficiency comparison of fasting plasma glucose combined with 1,5-anhydroglucitol and combined with glycated albumin in diabetes mellitus screening. Journal of Shanghai Jiaotong University(Medical Science). 2019; (12): 1077-1082. Chinese.
Non-enzymatic method for measuring GA	1	Shima K, Abe F, Chikakiyo H, Ito N. The relative value of glycated albumin, hemoglobin A1c and fructosamine when screening for diabetes mellitus. Diabetes Res Clin Pract. 1989 Nov 6;7(4):243-50. doi: 10.1016/0168-8227(89)90011-9.
Duplicate study population	1	Ma W-Y, Wu W-C, Wei J-N, Lin M-S, Shin S-R, Hua C-H, Liao Y-J, Chuang L-M, Li H-Y. When hemoglobin A1c fails: Serum glycated albumin to guide oral glucose tolerance tests in the diagnosis of diabetes mellitus. Diabetes. 2014; 1371-P: A343-A425.
Did not meet the research question or based on eligibility criteria	1	Kohzuma T, Koga M. Lucica GA-L glycated albumin assay kit: a new diagnostic test for diabetes mellitus. Mol Diagn Ther. 2010 Feb 1;14(1):49-51. doi: 10.1007/BF03256353.
	2	Furusyo N, Koga T, Ai M, Otokozawa S, Kohzuma T, Ikezaki H, Schaefer EJ, Hayashi J. Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS). Diabetologia. 2011 Dec;54(12):3028-36. doi: 10.1007/s00125-011-2310-6.
	3	Juraschek SP, Steffes MW, Miller ER 3rd, Selvin E. Alternative markers of hyperglycemia and risk of diabetes. Diabetes Care. 2012 Nov;35(11):2265-70. doi: 10.2337/dc12-0787.
	4	Juraschek SP, Steffes MW, Selvin E. Relationship between Nontraditional and Standard Markers of Glycemia. Circulation. 2012 Mar;125:AMP039. doi: https://doi.org/10.1161/circ.125.suppl_10.AMP039.
	5	Pan J, Zou J, Bao Y, Zhang L, Han J, Tang J, Ma X, Li Q, Jia W. Use of glycated albumin to distinguish occult diabetes mellitus from stress-induced hyperglycemia in Chinese orthopedic trauma patients. J Trauma Acute Care Surg. 2012 May;72(5):1369-74. doi: 10.1097/TA.0b013e3182464ba4.
	6	Hsu P, Ai M, Kanda E, Yu NC, Chen HL, Chen HW, Cheng MH, Kohzuma T, Schaefer EJ, Yoshida M. A comparison of glycated albumin and glycosylated hemoglobin for the screening of diabetes mellitus in Taiwan. Atherosclerosis. 2015 Sep;242(1):327-33. doi: 10.1016/j.atherosclerosis.2015.07.037.
	7	Sumner AE, Duong MT, Aldana PC, Ricks M, Tulloch-Reid MK, Lozier JN, Chung ST, Sacks DB. A1C Combined With Glycated Albumin Improves Detection of Prediabetes in Africans: The Africans in America Study. Diabetes Care. 2016 Feb;39(2):271-7. doi: 10.2337/dc15-1699.

	8	Park S, Lee W, Chung HS, Hong KS. Diagnostic Utility of Serum Glycated Albumin for Diabetes Mellitus and Its Correlation With Hyperlipidemia. Ann Lab Med. 2016 Jul;36(4):306-12. doi: 10.3343/alm.2016.36.4.306.
	9	Bellia C, Zaninotto M, Cosma C, Agnello L, Bivona G, Marinova M, Lo Sasso B, Plebani M, Ciaccio M. Clinical usefulness of Glycated Albumin in the diagnosis of diabetes: Results from an Italian study. Clin Biochem. 2018 Apr;54:68-72. doi: 10.1016/j.clinbiochem.2018.02.017.
Insufficient data for 2 × 2 contingency table	1	Yang C, Li H, Wang Z, Zhang W, Zhou K, Meng J, Zhao Y, Pan J, Lv X, Liang H, Jiang X. Glycated albumin is a potential diagnostic tool for diabetes mellitus. Clin Med (Lond). 2012 Dec;12(6):568-71. doi: 10.7861/clinmedicine.12-6-568.

Supplemental Table S3. Summary estimates of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes for all nine studies.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR– (95% CI)	DOR (95% CI)
Hwang, et al. 2014	210	63	106	473	0.66 (0.61 – 0.72)	0.88 (0.85 – 0.91)	5.65 (4.43 – 7.22)	0.38 (0.32 – 0.45)	14.87 (10.46 – 21.14)
Chume, et al. 2019	28	16	49	149	0.36 (0.26 – 0.48)	0.90 (0.85 – 0.94)	3.75 (2.16 – 6.51)	0.70 (0.59 – 0.84)	5.32 (2.66 – 10.65)
Zemlin, et al. 2019	61	78	33	1122	0.65 (0.54 – 0.74)	0.94 (0.92 – 0.95)	9.98 (7.69 – 12.96)	0.38 (0.29 – 0.49)	26.59 (16.42 – 43.05)
Wu, et al. 2016	63	26	69	1401	0.48 (0.39 – 0.57)	0.98 (0.97 – 0.99)	26.19 (17.20 – 39.89)	0.53 (0.45 – 0.63)	49.20 (29.34 – 82.49)
Li, et al. 2021	341	329	35	1230	0.91 (0.87 – 0.93)	0.79 (0.77 – 0.81)	4.30 (3.88 – 4.76)	0.12 (0.09 – 0.16)	36.42 (25.20 – 52.66)
Ikezaki, et al. 2015	18	56	11	91	0.62 (0.42 – 0.72)	0.62 (0.54 – 0.70)	1.63 (1.15 – 2.32)	0.61 (0.38 – 0.99)	2.66 (1.17 – 6.04)
Su, et al. 2018	226	59	109	297	0.67 (0.62 – 0.72)	0.83 (0.79 – 0.87)	4.07 (3.19 – 5.20)	0.39 (0.33 – 0.46)	10.44 (7.27 – 14.97)
He, et al. 2017	629	12	363	283	0.63 (0.60 – 0.66)	0.96 (0.93 – 0.98)	15.59 (8.94 – 27.18)	0.38 (0.35 – 0.42)	40.86 (22.61 – 73.86)
Ma, et al. 2010	580	281	175	935	0.77 (0.74 – 0.80)	0.77 (0.74 – 0.79)	3.32 (2.98 – 3.71)	0.30 (0.26 – 0.34)	11.03 (8.89 – 13.68)
Summary estimates and heterogeneity					**0.69 (0.68 – 0.71)** **X^2 = 201.77, (p<0.0001)** **I^2 = 96.0%**	**0.87 (0.86 – 0.87)** **X^2 = 552.14, (p<0.0001)** **I^2 = 98.6%**	**5.71 (4.00 – 8.15)** **Cochran-Q = 191.23, (p<0.0001)** **I^2 = 95.8%** **Tau-squared = 0.266**	**0.38 (0.30 – 0.49)** **Cochran-Q = 155.94, (p<0.0001)** **I^2 = 94.6%** **Tau-squared = 0.131**	**15.93 (9.81 – 25.87)** **Cochran-Q = 96.25, (p<0.0001)** **I^2 = 91.7%** **Tau-squared 0.484**

LR, likelihood ratio; DOR, diagnostic odds ratio; CI, confidence interval.



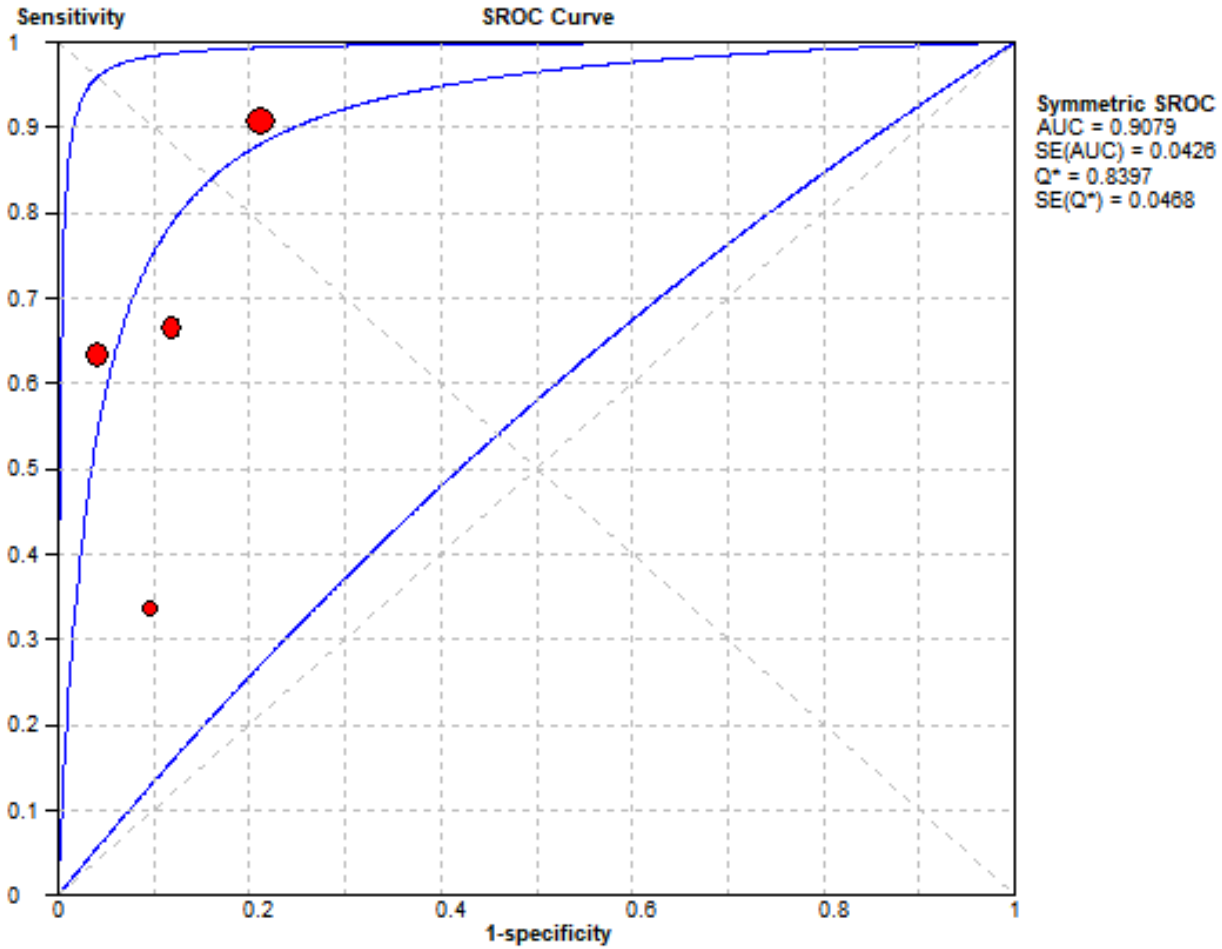
Supplemental Figure S1: Summary receiver operating characteristic curves (SROC) of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes for all nine studies.

Supplemental Table S4. Summary estimates of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes in studies with OGTT and/or HbA_{1c} as reference standard.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR– (95% CI)	DOR (95% CI)
Hwang, et al. 2014	210	63	106	473	0.66 (0.61 – 0.72)	0.88 (0.85 – 0.91)	5.65 (4.43 – 7.22)	0.38 (0.32 – 0.45)	14.87 (10.46 – 21.14)
Chume, et al. 2019	341	329	35	1230	0.34 (0.24 – 0.45)	0.90 (0.85 – 0.95)	3.51 (1.99 – 6.17)	0.73 (0.63 – 0.86)	4.78 (2.39 – 9.58)
Li, et al. 2021	29	15	57	141	0.91 (0.87 – 0.93)	0.79 (0.77 – 0.81)	4.30 (3.88 – 4.76)	0.12 (0.09 – 0.16)	36.42 (25.20 – 52.66)
He, et al. 2017	629	12	363	283	0.63 (0.60 – 0.66)	0.96 (0.93 – 0.98)	15.59 (8.94 – 27.18)	0.38 (0.35 – 0.42)	40.86 (22.61 – 73.86)
Summary estimates and heterogeneity					**0.68 (0.66 – 0.70)** **X^2 = 161.94, (p<0.0001)** **I^2 = 98.1%**	**0.84 (0.82 – 0.85)** **X^2 = 83.07, (p<0.0001)** **I^2 = 96.4%**	**5.89 (3.45 – 10.05)** **Cochran-Q = 41.64, (p<0.0001)** **I^2 = 92.8%** **Tau-squared = 0. 256**	**0.34 (0.19 – 0.60)** **Cochran-Q = 170.42, (p<0.0001)** **I^2 = 98.2%** **Tau-squared = 0.337**	**18.51 (8.26 – 41.47)** **Cochran-Q = 191.23, (p<0.0001)** **I^2 = 91.6%** **Tau-squared = 0.484**
Summary estimates and heterogeneity without Hwang, et al. 2014 (AUC 0.913)					**0.69 (0.66 – 0.71)** **X^2 = 161.3, (p<0.0001)** **I^2 = 98.8%**	**0.82 (0.81 – 0.84)** **X^2 = 71.4, (p<0.0001)** **I^2 = 97.2%**	**6.11 (2.10 – 17.77)** **Cochran-Q = 41.04, (p<0.0001)** **I^2 = 95.1%** **Tau-squared = 0.83**	**0.32 (0.13 – 0.82)** **Cochran-Q = 197.65, (p<0.0001)** **I^2 = 99%** **Tau-squared = 0.66**	**19.73 (6.05 – 64.31)** **Cochran-Q = 28.56, (p<0.0001)** **I = 93.0%** **Tau-squared = 1.00**
Summary estimates and heterogeneity without Li, et al. 2021 (AUC 0.905)					**0.62 (0.60 – 0.65)** **X^2 = 31.60, (p<0.0001)** **I^2 = 93.7%**	**0.91 (0.89 – 0.93)** **X^2 = 15.44, (p<0.0001)** **I^2 = 87.0%**	**6.72 (2.96 – 15.25)** **Cochran-Q = 20.13, (p<0.0001)** **I^2 = 90.1%** **Tau-squared = 0.46**	**0.47 (0.32 – 0.70)** **Cochran-Q = 53.45, (p<0.0001)** **I^2 = 96.3%** **Tau-squared = 0.116**	**14.49 (5.09 – 41.19)** **Cochran-Q = 22.84, (p<0.0001)** **I^2 = 91.2%** **Tau-squared = 0.77**
Summary estimates and heterogeneity without Chume, et al. 2019 (AUC 0.910)					**0.70 (0.68 – 0.72)** **X^2 = 116.33, (p<0.0001)** **I^2 = 98.3%**	**0.83 (0.82 – 0.85)** **X^2 = 76.65, (p<0.0001)** **I^2 = 97.4%**	**6.27 (3.55 – 13.31)** **Cochran-Q = 42.68, (p<0.0001)** **I^2 = 95.3%** **Tau-squared = 0.312**	**0.26 (0.14 – 0.49)** **Cochran-Q = 91.62, (p<0.0001)** **I^2 = 97.8%** **Tau-squared = 0.301**	**27.52 (13.89 – 54.54)** **Cochran-Q = 15.67, (p<0.0001)** **I^2 = 87.2%** **Tau-squared = 0.314**
Summary estimates and heterogeneity without He, et al. 2017 (AUC 0.901)					**0.75 (0.71 – 0.78)** **X^2 = 136.64, (p<0.0001)** **I^2 = 98.5%**	**0.82 (0.80 – 0.83)** **X^2 = 34.13, (p<0.0001)** **I^2 = 94.1%**	**4.61 (3.64 – 5.83)** **Cochran-Q = 5.31, (p<0.0001)**	**0.32 (0.11 – 0.94)** **Cochran-Q = 179.71, (p<0.0001)**	**14.31 (5.40 – 37.91)** **Cochran-Q = 29.25, (p<0.0001)**

			I^2 = 62.3% **Tau-squared = 0.02**	**I^2 = 98.9%** **Tau-squared = 0.873**	**I^2 = 93.2%** **Tau-squared = 0.679**

LR, likelihood ratio; DOR, diagnostic odds ratio; OGTT, oral glucose tolerance test; HbA_{1c}, glycated haemoglobin; AUC, area under the summary receiver operating characteristic curves; CI, confidence interval.

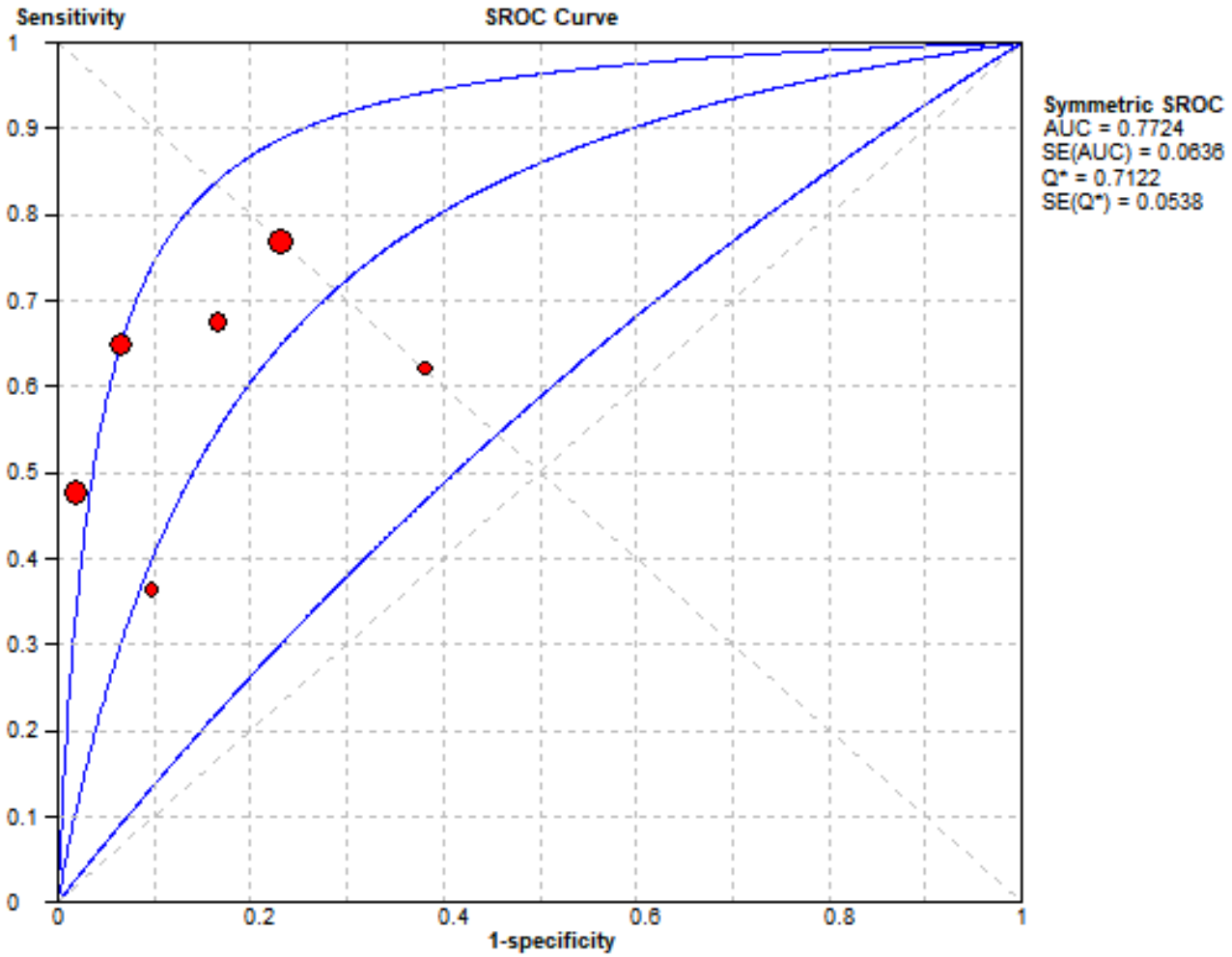


Supplemental Figure S2: Summary receiver operating characteristic curves (SROC) of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes in studies with OGTT and/or HbA_{1c} as reference standard. OGTT, oral glucose tolerance test; HbA_{1c}, glycated haemoglobin; AUC, area under the curve; CI, confidence interval.

Supplemental Table S5. Summary estimates of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes in studies with OGTT as reference standard.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR– (95% CI)	DOR (95% CI)
Chume, et al. 2019	28	16	49	149	0.36 (0.26 – 0.48)	0.90 (0.85 – 0.94)	3.75 (2.16 – 6.51)	0.70 (0.59 – 0.84)	5.32 (2.66 – 10.65)
Zemlin, et al. 2019	61	78	33	1122	0.65 (0.54 – 0.74)	0.94 (0.92 – 0.95)	9.98 (7.69 – 12.96)	0.38 (0.29 – 0.49)	26.59 (16.42 – 43.05)
Wu, et al. 2016	63	26	69	1401	0.48 (0.39 – 0.57)	0.98 (0.97 – 0.99)	26.19 (17.20 – 39.89)	0.53 (0.45 – 0.63)	49.20 (29.34 – 82.49)
Ikezaki, et al. 2015	18	56	11	91	0.62 (0.42 – 0.72)	0.62 (0.54 – 0.70)	1.63 (1.15 – 2.32)	0.61 (0.38 – 0.99)	2.66 (1.17 – 6.04)
Su, et al. 2018	226	59	109	297	0.67 (0.62 – 0.72)	0.83 (0.79 – 0.87)	4.07 (3.19 – 5.20)	0.39 (0.33 – 0.46)	10.44 (7.27 – 14.97)
Ma, et al. 2010	580	281	175	935	0.77 (0.74 – 0.80)	0.77 (0.74 – 0.79)	3.32 (2.98 – 3.71)	0.30 (0.26 – 0.34)	11.03 (8.89 – 13.68)
Summary estimates and heterogeneity					**0.69 (0.66 – 0.71)** **X^2 = 84.69, (p<0.0001)** **I^2 = 94.1%**	**0.89 (0.88 – 0.89)** **X^2 = 436.26, (p<0.0001)** **I^2 = 98.6%**	**5.25 (2.86 – 9.63)** **Cochran-Q = 160.29, (p<0.0001)** **I^2 = 96.9%** **Tau-squared = 0.543**	**0.46 (0.34 – 0.62)** **Cochran-Q = 74.10, (p<0.0001)** **I^2 = 93.3%** **Tau-squared = 0.126**	**11.91 (6.42 – 22.10)** **Cochran-Q = 59.36, (p<0.0001)** **I^2 = 91.6%** **Tau-squared = 0.523**
Summary estimates and heterogeneity without Chume, et al. 2019 (AUC 0.786)					**0.70 (0.68 – 0.73)** **X^2 = 48.77, (p<0.0001)** **I^2 = 91.8%**	**0.88 (0.88 – 0.89)** **X^2 = 435.72, (p<0.0001)** **I^2 = 99.1%**	**5.59 (2.83 – 11.06)** **Cochran-Q = 160.21** **I^2 = 97.5%** **Tau-squared = 0.583**	**0.41 (0.32 – 0.54)** **Cochran-Q = 32.49** **I^2 = 87.7%** **Tau-squared = 0.068**	**13.78 (7.03 – 27.04)** **Cochran-Q = 53.21** **I^2 = 92.5%** **Tau 0.526**
Summary estimates and heterogeneity without Ikezaki, et al. 2015 (AUC 0.817)					**0.69 (0.66 – 0.71)** **X^2 = 84.12, (p<0.0001)** **I^2 = 95.2%**	**0.89 (0.89 – 0.90)** **X^2 = 363.13, (p<0.0001)** **I^2 = 98.9%**	**6.64 (3.43 – 12.84)** **Cochran-Q = 133.76** **I^2 = 97.0%** **Tau-squared = 0.535**	**0.44 (0.32 – 0.61)** **Cochran-Q = 72.18** **I2 = 94.5%** **Tau 0.129**	**15.30 (8.33 – 28.10)** **Cochran-Q = 44.90** **I^2 = 91.1%** **Tau-squared = 0.423**
Summary estimates and heterogeneity without Chume, et al. 2019 and Ikezaki, et al. 2015 (AUC 0.825)					**0.71 (0.68 – 0.73)** **X^2 = 47.81, (p<0.0001)** **I^2 = 93.7%**	**0.89 (0.88 – 0.90)** **X^2 = 363.00, (p<0.0001)** **I^2 = 99.2%**	**7.56 (3.53 – 16.19)** **Cochran-Q = 133.54** **I2 = 97.8%** **Tau-squared = 0.583**	**0.39 (0.30 – 0.51)** **Cochran-Q = 28.88** **I2 = 89.6%** **Tau-squared = 0.065**	**19.12 (9.92 – 36.85)** **Cochran-Q = 37.82** **I2 = 92.1%** **Tau-squared = 0.405**

LR, likelihood ratio; DOR, diagnostic odds ratio. OGTT, oral glucose tolerance test.

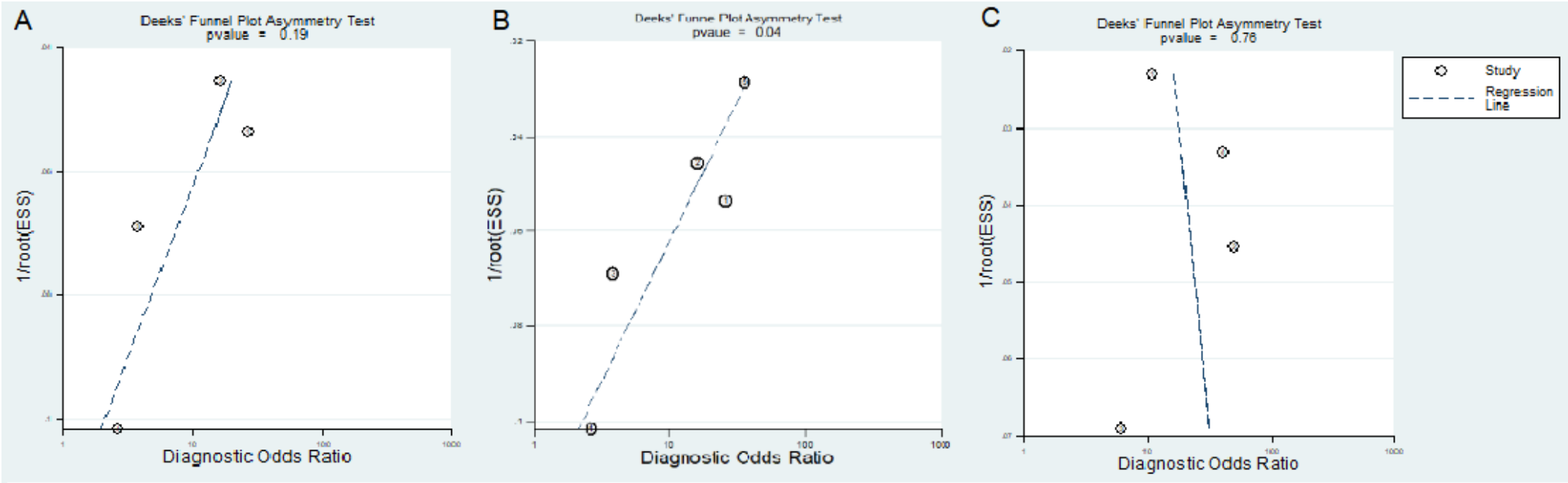


Supplemental Figure S3: Summary receiver operating characteristic curves (SROC) of glycated albumin at designated optimal cutoffs for the diagnosis of diabetes in studies with OGTT as reference standard. OGTT, oral glucose tolerance test.

Supplemental Table S6. Pooled sensitivity, specificity, LR+, LR-, DOR and AUC of GA

Pooled indexes	**GA of 15%**	**GA of 17.1%**
Sensitivity	0.671 (95% CI 0.605 – 0.730)	0.551 (95% CI 0.367 – 0.722)
I^2 (P-value)	32.1% (0.22)	97.3% (<0.0001)
Specificity	0.809 (95% CI 0.648 – 0.906)	0.944 (95% CI 0.853 – 0.979)
I^2 (P-value)	98.0% (<0.0001)	99.2% (<0.0001)
LR+	3.51 (95% CI 1.74 – 7.05)	9.78 (95% CI 4.29 – 22.34)
I^2 (P-value)	97.0% (<0.0001)	97.5% (<0.0001)
LR–	0.40 (95% CI 0.30 – 0.54)	0.47 (95% CI 0.33 – 0.69)
I^2 (P-value)	72.7% (0.01)	97.3% (<0.0001)
DOR	8.61 (95% CI 3.36 – 22.07)	20.56 (95% CI 9.01 – 46.94)
I^2 (P-value)	92.9% (<0.0001)	93.1% (<0.0001)
AUC	0.72 (95% CI 0.68 – 0.75)	0.85 (95% CI 0.82 – 0.88)
Q* value	0.6590	0.7775

LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio; ROC, receiver operating characteristic; AUC, area under hierarchical summary receiver operating characteristic curves



Supplemental Figure S4: Deeks' funnel plot for publication bias. (A) GA ≥15% to diagnose diabetes by OGTT; (B) GA ≥15% to diagnose diabetes regardless of the reference test: OGTT solely or OGTT and/or HbA_{1c}; (C) GA ≥17.1% to diagnose diabetes regardless of the reference test: OGTT solely or OGTT and/or HbA_{1c}. ESS, effective sample size; GA, glycated albumin; OGTT, oral glucose tolerance test; HbA_{1c}, glycated haemoglobin.

Supplemental Table S7. Summary estimates of GA ≥17.1% compared with other reports summary estimates of HbA_{1c} ≥6.5% for the diagnosis of diabetes.

Study [reference]	Index test and cut-off	AUC	DOR	Sensitivity	Specificity	LR+	LR–
Present study	GA ≥17.1%	0.85	20.7	0.551	0.944	9.78	0.47
Xu et al. [34]	HbA_{1c} ≥6.5%	0.93	40.6	0.518	0.956	19.0	0.48
Kaur et al [35]	HbA_{1c} ≥6.5%	NA	NA	0.50	0.973	18.32	0.51
NCD-RisC group [36]	HbA_{1c} ≥6.5%	NA	NA	0.305	0.997	NA	NA
Hoyer et al [37]	HbA_{1c} ≥6.5%	NA	NA	0.684	0.959	NA	NA

GA, glycated albumin; HbA_{1c}, glycated haemoglobin; AUC, area under the summary receiver operating characteristic curves; DOR, diagnostic odds ratio; LR, likelihood ratio; NA, not available.

Capítulo 4

Full title: **Is there a role for Glycated Albumin in the Diagnosis of Gestational Diabetes Mellitus?**

Short title: **Glycated Albumin & Gestational Diabetes Mellitus**

Fernando Chimela Chume[1, 2#]

Paula Breitenbach Renz[1#]

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HIGHLIGHTS

- Previously studies in general population reported that glycated albumin (GA) presents similar accuracy as HbA1c for detecting diabetes.
- The present study showed that in pregnant women the area under the curve (AUC) for GA in the diagnosis of gestational diabetes mellitus (GDM) was much lower than AUC for HbA1c.
- Unlike HbA1c, GA does not have the ability to correctly discriminate those with and without GDM.
- Our findings may provide a cautious approach when assessing glycaemic status using GA in pregnant women, since pregnancy can be considered a confounding factor.

ABSTRACT

Background - Studies in the general population have advocated glycated albumin (GA) as a useful alternative to glycated haemoglobin (HbA1c) under conditions wherein the latter does not reflect glycaemic status accurately. There are few studies in other populations, especially in pregnant women. Therefore, the aim of this study was to assess the clinical utility of GA in the diagnosis of gestational diabetes mellitus (GDM).

Materials and methods - This diagnostic test accuracy study was performed in 149 Brazilian women at 24-28 weeks of gestation referred for an oral glucose tolerance test (OGTT) in a tertiary university hospital. Receiver Operating Characteristic (ROC) curves were used to access the performance of GA and HbA1c in the diagnosis of GDM by the reference OGTT.

Results - GDM by OGTT (IADPSG criteria) was detected in 18.8% of participants. According to ROC analysis, the area under the curve (AUC) for GA was 0.531 (95% CI: 0.405 – 0.658, p =0.065) lower than that for HbA1c [0.743 (95% CI: 0.636 – 0.849; p=<0.001] for the detection of GDM (p=0.004). The equilibrium cut-off value for GA was 12.6%; sensitivity and specificity in this cut-off point were 53.6% and 54.2%, respectively.

Conclusions - GA at 24–28 weeks of gestation does not have ability to correctly discriminate those with and without GDM. In summary, the lack of sensitivity found in our results do not support the solely use of GA in the diagnosis of GDM.

Key words: Gestational diabetes mellitus, Glycated Albumin, HbA1c, Oral glucose tolerance test, Diagnostic accuracy.

LIST OF ABBREVIATIONS:

GDM, gestational diabetes mellitus; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; 1h-PG - 1-h plasma glucose after a 75-g OGTT; 2h-PG - 2-h plasma glucose after a 75-g OGTT; HbA1c, glycated haemoglobin, GA, glycated albumin; GSP, glycated serum proteins; ROC, receiver operating characteristic; AUC, area under the ROC curve; SD, standard deviation; LR, likelihood ratio; WHO, World Health Organization; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; STARD, Standard for Reporting Diagnostic Accuracy; HCPA, Hospital de Clinicas de Porto Alegre.

INTRODUCTION

Diabetes that is first diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation is named gestational diabetes mellitus (GDM) [1]. This condition carries adverse effects for the mother and neonate. The detection and treatment of this condition may reduce the risk of adverse maternal, fetal, and neonatal outcomes [2–4]. Tough GDM may be asymptomatic and many people do not have the classic diabetes risk factors. Therefore, the World Health Organization (WHO) recommends the screening of GDM for all pregnant women [5].

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, a large-scale multinational cohort study, demonstrated that risk of adverse maternal, fetal, and neonatal outcomes continuously increased with maternal glycemia at 24–28 weeks of gestation, even within ranges previously considered normal for pregnancy [6]. These results support the need for screening for GDM between the 24th and 28th week of gestation [1]. However, the lack of threshold for risk in most complications led to great controversy about the diagnostic criteria for GDM. Different diagnostic criteria will identify different degrees of maternal hyperglycemia and maternal/fetal/neonatal risk, leading to conflicted recommendations from experts on optimal strategies for the diagnosis of GDM [1]. GDM diagnosis may be based in one-step 75-g OGTT [7] or two-step approach with a 50-g OGTT screening followed by a 100-g OGTT for those who screened positive [8]. The Brazilian Diabetes Society [9] recommends International Association of the Diabetes and Pregnancy Study Groups (IADPSG) strategy which is also adopted by WHO.

Of late, HbA1c, the current standard test for monitoring glycaemic control, is also considered as diagnostic tool for diabetes in the general population [1]. Recent studies examine the validity of HbA1C in different population, including among pregnant women. A systematic review and meta-analysis that examined the overall accuracy of HbA1c in the diagnosis of GDM showed that HbA1c presented high specificity but low sensitivity regardless of the threshold used to diagnose GDM [10]. HbA1c performance in pregnant women is similar to the one reported to HbA1c when it is used as diagnostic tool for diabetes in general population [1]. Although advantages of HbA1c over glucose-based tests includes patient’s comfort (fasting not required) and measurement stability, there are some situations that HbA1c is not suitable to use like conditions with altered blood red cell turnover, such as

anaemia [11], a common condition in pregnant women. Therefore, it is important to consider alternative procedures for the diagnosis of GDM.

Glycated albumin (GA) is a test that has gained prominence as an alternative glycaemic marker [12]. GA is a measure of glycaemia based on the amount of glucose in serum or plasma attached to albumin, rather than to haemoglobin. The assay is well standardised and has been automated for high throughput analysis. GA reflects short-term mean glycaemia (2–3 weeks), rather than 2–3 months mean glycaemia observed for HbA1c [12]. Like HbA1c, GA correlates well with diabetic complications, and even death in people with diabetes [13, 14].

Additionally, GA is haemoglobin/erythrocyte independent and the performance of the test in the diagnosis of diabetes is similar to HbA1c in most studies [15–20]. Therefore, those studies advocate GA as a useful alternative to HbA1c under conditions wherein the latter does not reflect glycaemic status accurately.

Although evidence about GA performance in diagnosis and screening of diabetes have been available in general populations, few studies exist in other populations, especially in pregnant women [21, 22].

Then, the current study was designed to assess the clinical utility of GA in the diagnosis of GDM.

MATERIALS AND METHODS

This cross-sectional study of diagnostic accuracy was designed and reported according to Standard for Reporting Diagnostic Accuracy (STARD) initiative guidelines [23].

Participants

This study includes pregnant women in 24-28 weeks of gestation that were referred to perform OGTT in Hospital de Clinicas de Porto Alegre (HCPA) between September 2009 and July 2012. All participants were previously included in a study evaluating the use of HbA1c for the diagnosis of GDM. During this previous study they signed an informed consent term and provided clearance for the use of stored material and data in related future studies [24]. The present study protocol was approved by the Ethics Committee of the Hospital de Clinicas de Porto Alegre (GPPG-HCPA) and is registered by the number GPPG 2018-0409.

Exclusion criteria for this study were pregnant women under 18 years old, presence of twin pregnancy, women with established diagnosis of diabetes or who were receiving anti-diabetic medication, presence of clinical conditions known to interfere or lead to misinterpretation of GA and/or HbA1c results, such as albumin levels <3.0 g/dl, severe anaemia (haemoglobin <7 g/dL), presence of variant haemoglobin, recent transfusion, rheumatic disorder, hepatic cirrhosis, nephrotic syndrome, chronic kidney disease, untreated thyroid dysfunction, and/or Cushing syndrome [11, 12].

Glycaemic status was defined according to recommendations of American Diabetes Association using one step 75g OGTT strategy - IADPSG criteria [1]. GDM was defined by: *(a)* fasting plasma glucose (FPG) ≥92mg/dL and/or (*b*) 1h plasma glucose after ingestion of 75g of glucose (1h-PG) ≥180mg/dL and/or *(c)* 2h plasma glucose after ingestion of 75g of glucose (2h-PG) ≥180mg/dL and/or 2h ≥153mg/dL.

Laboratory Analysis

All pregnant women underwent a standard 75g OGTT after an overnight fast of at least 8 hours. Blood samples for glucose determination were collected by venipuncture into tubes containing sodium fluoride at fasting, 1-hour and 2-hour after 75g glucose oral intake. Plasma glucose concentrations were measured by

colorimetric enzymatic method in the biochemistry automated analyser Cobas® c702 (Roche Diagnostics, Germany).

HbA1c were measured in K2EDTA-anticoagulated whole blood by high performance liquid chromatography (HPLC) using VARIANT II™ System (BioRad Laboratories, Hercules, CA, USA). This HbA1c assay is certified by the National Glycohemoglobin Standardization Program and aligned to the DCCT reference and the International Federation of Clinical Chemistry reference [11]. The inter-assay coefficient of variation (CV) for HbA1c method was <3.0%.

Fasting serum samples were stored at -80°C until they were used for measurement of GA. GA was determined by an enzymatic method (GlycoGap®, Diazyme Laboratories, Poway, CA) in the automated analyser Cobas® c702 (Roche Diagnostics, Germany), previously validated in our laboratory [25] and the inter-assay CV for this assay was 3.0%. Total albumin was measured with bromocresol green colorimetric method. GlycoGap® GA assay quantifies the total of glycated serum proteins (GSP, µmol/L), which are converted to percent of GA by the following conversion equation: GA (%) = {[GSP (µmol/L) x 0.182 + 1.97]/total albumin (g/dL)} + 2.9 [25].

Statistical Analysis

Sample size was calculated based on the results obtained in studies in the general population, where GA has accuracy similar to that of HbA1c [15-20]. Considering a significance level of 5%, power of 80% and an area on the expected curve of 0.714, as found in previous study during evaluation of the performance of the HbA1c test to detect GDM [24], the total sample size of 54 individuals was reached, 27 in each group. Adding 10% for possible losses and refusals, the sample size would require 60 participants. Sample size calculations were carried out in PSS Health tool online version [26].

Data are expressed as mean ± standard deviation (SD) or frequencies (%). Data normality was examined using histograms and Shapiro-Wilk test. Student's T-tests and chi-squared were used as appropriate. Pearson's correlation coefficients were calculated to assess correlations between GA and FPG, 1h-PG, 2h-PG and HbA1c. Receiver Operating Characteristic (ROC) curve was used to analyze the performance of the HbA1c test to diagnose GDM considering the OGTT as

reference diagnostic criteria. All areas under the curves (AUC) were pairwise compared by DeLong's test.

The IBM SPSS software for Windows, version 20.0 (Statistical Package for Social Sciences—Professional Statistics, IBM Corp, Armonk, USA) and MedCalc, version 19.1 (MedCalc software, Ostend, Belgium) were used for data analysis. P values less than 0.05 were considered significant.

RESULTS

A total of 149 pregnant women between 24 and 28 weeks of gestation and without pre-existing diabetes were included in this study. Twenty-eight (18.8%) participants were diagnosed with GDM using OGTT as diagnostic criteria. The characteristics of these participants are shown in Table 1. Participants with GDM were older and had higher values of FPG, 1hPG, 2hPG and HbA1c. No significant difference was detected in GA levels between the two groups.

GA significantly correlated with HbA1c only on pregnant women with GDM (women with GDM: r =0.405, p =0.033 and women without DM: r =-0.081, p =0.379). Whereas GA did not correlate significantly with FPG, 1hPG and 2hPG; HbA1c correlated significantly with FPG and 2hPG on women without GDM (r =0.294, p =0.001 and r =0.279, p =0.002, respectively). Correlations between GA, HbA1c, FPG, 1hPG and 2hPG by GDM status are presented in Table 2.

The performances of GA, HbA1c and FPG for the diagnosis of GDM by the OGTT are shown in Figure 1. According to ROC analysis (Fig. 1), the overall accuracy of GA to diagnose GDM is very low showing that GA does not have ability to correctly discriminate those with and without GDM. The AUC for GA was 0.531 (95% CI: 0.405 – 0.658; p =0.607). The equilibrium cut-off value for GA was 12.7%; sensitivity and specificity in this cut-off point were 53.6% and 53.3%, respectively (Table 3). GA ≥12.7% yielded LR+ and LR- of 1.15 and 0.87, respectively. However, GA higher than 15% showed very high specificity (> 98%) to identify GDM, with LR+ and LR- of 2.14 and 0.98, respectively. The AUC for HbA1c was 0.743 (95% CI: 0.636 – 0.849; p <0.001). The difference between AUC for GA and HbA1c was 0.212 (95% CI: 0.068 – 0.355; p <0.004). FPG had the highest AUC [0.865 (95% CI: 0.772 – 0.958; p <0.001)] for the detection of GDM than the AUCs

of GA and HbA1c, though the difference between AUC for FPG and HbA1c was not statistically significant [0.122 (95% CI: 0.010 – 0.254; p <0.070)].

DISCUSSION

In this study, we evaluated the performance of GA in the diagnosis of GDM by OGTT as the reference test. Our results showed that GA has a poor overall accuracy to diagnose GDM without the ability to correctly discriminate women with and without GDM.

Our study is in agreement with two previous studies that also reported that GA was not suitable as a diagnostic tool for GDM [21, 22]. In the first study, a cross-sectional case-control study [21], examined 80 Turkish pregnant women and reported AUC of 0.550, similar to the AUC in our study. In the second study, that examined 665 Chinese pregnant women [22], the AUC for the detection of GDM was 0.568. Similar to the Chinese study [22], our findings showed that FPG has a higher diagnostic value than GA and HbA1c for the detection of GDM. On the other hand, the results of Saglam *et al*. study reported no difference in the AUCs of GA and HbA1c [21]. By contrast, the present study found that AUC for GA in the diagnosis of GDM by the OGTT was much lower than for HbA1c (p=0.004).

Studies performed in the general population have usually reported better accuracy of GA in the diagnosis of diabetes with AUCs ranging from 0.70 and 0.90 [15–20]. This performance is similar to that of HbA1c in most studies. Therefore, these studies have advocated that GA may be a useful alternative to HbA1c under conditions wherein the latter does not reflect glycaemic status accurately.

GA is a measure of glycaemia based on the amount of glucose present in the blood attached to albumin, rather than to haemoglobin. Thus, GA is haemoglobin/erythrocyte independent, consequently, its measurement appears to be more appropriate in people with anaemia, a condition usually seen in pregnant women.

In this study, GA was found to be associated only with HbA1c in women with GDM. GA levels were not associated with glycaemic tests (FPG, 1hPG and 2hPG). In contrast, HbA1c was associated with FPG and 2hPG in women without GDM. These results may suggest that, in general, pregnancy may be considered as a confounding factor when assessing glycaemic status using GA. Yi et al. already reported that GA levels decreased as pregnancy progress with or without GDM [26].

Hiramatsu et al. had shown similar results in healthy pregnant women [28]. Li et al. also related that GA levels reduce continually as pregnancy progressed in both women with and without GDM [29]. Nonetheless, they observed that elevated GA levels had a positive association with the incidence of babies with birthweights ≥3.5 g and macrosomia in GDM women with poor glycaemic control [29]. In two similar studies, Mendes et al. showed that GA, besides from providing additional information to HbA1c, when used separately, performed better than traditional biomarkers in predicting neonatal birthweight and large-for-date babies in pregnant women with GDM [30, 31]. Caution should therefore be advised in interpreting GA measurements during pregnancy. The reasons why and how GA decreases from early to late pregnancy are yet to be elucidated.

Although this is out of the scope of this study, we analyzed our data to explore the association of GA and body weigth. There was no association between GA and current body weigth or body mass index in our pregnant women (result not shown). We think this topic is clinically relevant and properly designed studies are needed to evaluate the influencing factors of GA in pregnant women.

The present study has some strength. As far as we know is the first to relate the performance of GA to diagnose GDM in Brazilian women. Women with disorders that could interfere on albumin metabolism and influence on GA levels were excluded. We attempted to follow the STARD 2015 reporting guideline for diagnostic accuracy studies to assure reporting the results adequately. The study also has limitations. The sample size is small, but it was calculated a priori to assure the study power of 80% and an estimated alfa error of 5%. Besides, patients were consecutively enrolled, and the sample reflects the prevalence of GDM in our population, as recommended in diagnostic accuracy study [32]. We use a single dosage of GA to ascertain whether measurement of GA can be employed to diagnose GDM, however, the chosen period at 24–28 weeks of gestation matches with the period of increased insulin resistance caused by placental hormones. In addition, we were not able to evaluate any relationship between GA levels and neonatal complications due to the cross-sectional study design.

In conclusion, serum GA at 24–28 weeks of gestation does not have enough diagnostic accuracy to correctly discriminate those with and without GDM. In summary, our results do not support the solely use of GA at 24–28 weeks of gestation in the diagnosis of GDM. The potential of GA in pregnancy remains

unknown. Further studies are necessary to determine the value of testing GA in pregnant women and the effects of pregnancy on GA merit consideration when using GA as an indicator of glycaemic control in clinic.

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COMPLIANCE WITH ETHICAL STANDARDS:

Conflict of interest:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval:

The present study protocol followed the institutional policies that are in compliance with the Declaration of Helsinki. The protocol was approved prior to commencing by the Ethics Committee of the Hospital de Clinicas de Porto Alegre (GPPG-HCPA) (registration number GPPG 2018-0409).

Informed consent and consent for publication:

Informed consent providing clearance for the use of stored material, data and their publication after anonymization was obtained from all individual participants included in the study.

DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author on request.

AUTHOR CONTRIBUTIONS:

Conceived and designed the experiments: FCC, PBR, JLC. Contributed materials and reagents: FCC, PBR, JLC. Performed the experiments: FCC, PBR, MKH, PACF. Acquired the data: PBR, MKH. Analysed and interpreted the data: FCC, PBR, JLC. Drafted the article/wrote the paper: FCC, PBR, JLC. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

FCC and PBR should be considered joint first author.

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Table 1. Clinical and laboratory characteristics of participants in the study.

Characteristics	All participants (n = 149)	Without GDM (n=121)	With GDM (n=28)	p-value
Age (years)	28.5 ± 6.6	27.9 ± 6.7	30.7 ± 5.8	<0.05
Gestational age (weeks)	26.5 ± 4.6	26.7 ± 4.2	25.6 ± 6.3	0.239
Systolic BP (mmHg)	113.7 ± 12.9	105.3 ± 29.4	114.6 ± 30.1	0.185
Diastolic BP (mmHg)	70.7 ± 11.2	71.4 ± 13.3	76.4 ± 12.0	0.115
HbA1c [mmol/mol; (%)]	32 ± 4.4 (5.1 ± 0.4)	32 ± 3.3 (5.1 ± 0.3)	36 ± 4.4 (5.4 ± 0.4)	<0.05
Glycated albumin (%)	12.7 ± 1.2	12.7 ± 1.2	12.7 ± 1.5	0.852
FPG (mg/dL)	81.4 ± 8.4	78.9 ± 5.6	92.2 ± 9.9	<0.05
1hPG (mg/dL)	130.3 ± 35.2	119.1 ± 25.6	180.2 ± 28.1	<0.05
2hPG (mg/dL)	114.9 ± 28.1	127.4 ± 36.4	150.39 ± 29.5	<0.05
Serum albumin (g/l)	3.7 ± 1.3	3.7 ± 0.5	3.8 ± 0.4	0.236
Haemoglobin (g/l)	11.7 ± 0.9	11.7 ± 0.9	12.1 ± 0.8	<0.05
Haematocrit (%)	34.7 ± 2.8	34.4 ± 2.8	35.7 ± 2.4	<0.05

Data are expressed as mean ± SD or frequencies; BP, blood pressure; GDM, gestational diabetes mellitus; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; 1hPG, plasma glucose 1h after oral glucose; 2hPG, plasma glucose 2h after oral glucose. GDM was defined as FPG ≥92 mg/dL and/or 1hPG ≥180 mg/dL and/or 2hPG ≥153 mg/dL after an oral glucose tolerance test.

Table 2. Correlations of GA, HbA_{1c}, FPG and 2hPG by GDM status

A. Pregnant women without GDM (n = 121)					
	GA	FPG	1hPG	2hPG	HbA1c
GA	1	-0.012	-0.089	-0.017	-0.081
FPG		1	0.194[a]	0.275[b]	0.294[b]
1hPG			1	0.045	0.162
2hPG				1	0.279[b]
HbA1c					1
B. Pregnant women with GDM (n = 28)					
	GA	FPG	1hPG	2hPG	HbA1c
GA	1	0.209	-0.049	0.069	0.405[a]
FPG		1	0.124	-0.146	0.378[a]
1hPG			1	0.053	-0.390[a]
2hPG				1	0.209
HbA1c					1

[a] Correlation is significant at the 0.01 level (2-tailed). [b] Correlation is significant at the 0.05 level (2-tailed). FPG, fasting plasma glucose; 1hPG, plasma glucose 1 h after oral glucose; 2hPG, plasma glucose 2 h after oral glucose; HbA1c, glycated haemoglobin; GA, glycated albumin; GDM, gestational diabetes mellitus.

Table 3. Performance of different cut-offs of GA, HbA1c and FPG to diagnose GDM. (n=149)

	Threshold	Sensitivity (%)	Specificity (%)
GA (%)	12.0	71.4	29.2
	12.7	53.6	53.3
	13.0	42.9	61.7
	13.5	28.6	77.5
	14.0	21.4	88.3
	14.5	10.7	94.2
	15.0	3.6	98.3
	15.2	3.6	99.2
HbA1c [mmol/mol; (%)]	26 (4.5)	100.0	5.0
	31 (5.0)	78.6	48.8
	37 (5.5)	50.0	86.8
	45 (6.3)	3.6	100
FPG (mg/dl)	80.5	85.7	59.5
	85.5	75.0	90.1
	89.5	64.3	97.5
	92.5	57.1	100
	95.5	39.3	100

GDM, gestational diabetes mellitus; HbA1c, glycated haemoglobin; GA, glycated albumin; FPG, fasting plasma glucose.

FIGURE LEGEND

Fig. 1: Receiver operating characteristic (ROC) curves to access the performance of FPG, GA and HbA_{1c} in the diagnosis of GDM by OGTT. The AUC value for FPG was 0.865 (SE: 0.048, 95% CI: 0.772 – 0.958, p <0.001), GA was 0.531 (SE: 0.065, 95% CI: 0.405 – 0.658, p =0.607) and for HbA1c was 0.743 (SE: 0.054, 95% CI: 0.636 – 0.849, P <0.001). GDM, gestational diabetes mellitus; HbA1c, glycated haemoglobin; GA, glycated albumin; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; AUC, area under the ROC curve; SE, standard error; CI, confidence interval.

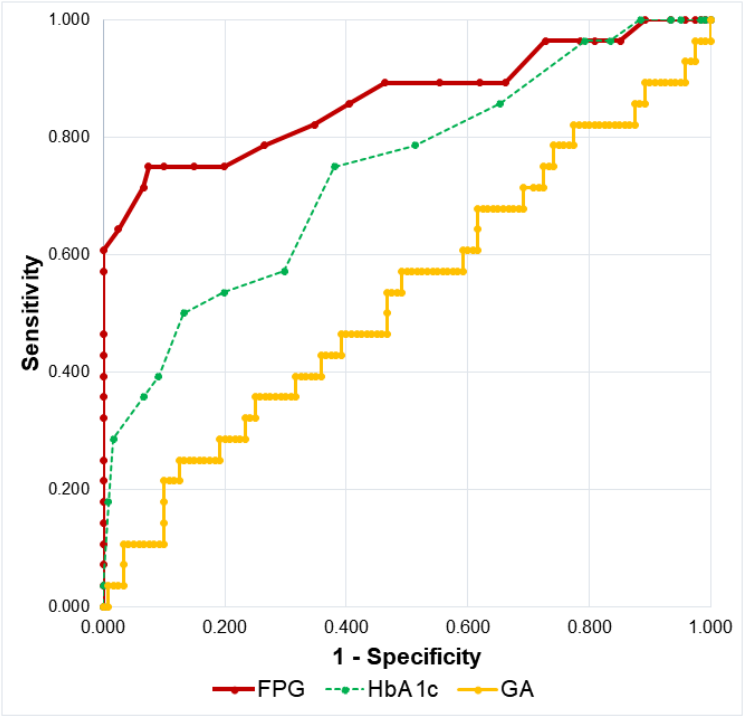
1.000
0.800
0.600
0.400
0.200
0.000
Sensitivity
0.000
0.200
0.400
0.600
0.800
1.000
1 - Specificity
FPG
HbA1c
GA

Figure 1

CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Para esta tese, avaliamos o desempenho da AG como marcador glicêmico em diferentes contextos clínicos. Para tanto, quatro estudos com objetivos distintos foram realizados.

O primeiro artigo dessa tese consiste em uma revisão sistemática com meta-análise sobre a acurácia da AG no diagnóstico de diabetes mellitus na população geral. A AG apresentou boa acurácia diagnostica para diabetes. O efeito do ponto de corte de AG na acurácia diagnóstica foi relatado em AG de 15,0% e 17,1%. Dentre eles, AG de 17,1% foi o ponto de corte ideal para diagnosticar diabetes em população geral com moderada sensibilidade [55,1% (95% CI 36,7–72,2%)] e alta especificidade [94,4% (95% CI 85,3–97,9%)]. Novos estudos de acurácia de AG no diagnóstico de diabetes que relatam dados de sensibilidade e especificidade de vários pontos de corte de AG são necessários para determinar o efeito dos pontos de corte de AG e definir qual o melhor ponto de corte de AG no diagnóstico de diabetes em população geral.

No segundo artigo avaliou-se o desempenho da AG no momento da admissão para detectar anormalidades glicêmicas em indivíduos hospitalizados pela COVID-19. Nesse estudo a AG apresentou desempenho moderado à ótimo para identificar adultos com diabetes prévio não diagnosticado pré-admissão, diabetes não controlado e hiperglicemia intra-hospitalar que necessitou de prescrição de terapia com insulina. Os pontos de corte da AG adequados para detectar essas condições apresentavam alta especificidade e baixa a moderada sensibilidade, o que sugere que a AG no momento da admissão pode ser útil para identificar adultos com essas condições durante hospitalização por COVID-19. Entretanto, em indivíduos sem evidência de diabetes prévia, AG não foi precisa na identificação de hiperglicemia da admissão definida pela primeira glicemia aleatória na admissão. Este é o primeiro estudo na literatura sobre a acurácia da AG durante hospitalização por COVID-19, e outros estudos em diferentes populações são necessários.

Também com objetivo de entender melhor o papel de AG durante a hospitalização por COVID-19 temos dois estudos de coorte em andamento. O primeiro uma análise longitudinal de dados para avaliar se a AG no momento da admissão está associada a desfechos clínicos adversos, como risco de

exacerbação com necessidade de ventilação mecânica e/ou admissão em UTI e mortalidade hospitalar. O segundo analisamos se AG pode ser útil para monitorar as variações de curto prazo do controle glicêmico durante a hospitalização por COVID-19.

No terceiro trabalho avaliou-se a acurácia da AG no diagnóstico de DMG. Em concordância com achados existentes na literatura, nesse estudo AG na 24ª–32ª semana de gestação apresentou baixa sensibilidade para DMG, sem capacidade de discriminar gestantes com e sem DMG.

Por fim, no quarto artigo avaliou-se a relação do estado glicêmico definido por TOTG, e níveis de HbA1c e AG com desfechos adversos da gravidez em gestantes com e sem DMG. Nesse estudo AG na 24ª–32ª semana de gestação apresentou baixo valor preditivo de risco para desfechos perinatais adversos em gestantes. As possíveis complicações da hiperglicemia na gestação, como macrossomia, cesariana, distocia de ombro, óbito intrauterino, prematuridade e internação em unidade de terapia intensiva neonatal, também não ocorreram de forma mais frequente naqueles que tiveram DMG diagnosticado por TOTG.

Com base nos achados desta tese, o uso de AG pode ampliar o diagnóstico precoce de diabetes na população geral, alinhado com o que já é observado nos testes de rotina que não necessariamente detectam o diabetes nos mesmos indivíduos. A AG no momento da admissão hospitalar também pode ser utilizado para detectar anormalidades glicêmicas durante a hospitalização por COVID-19. No entanto, não houve benefício em utilizar o teste AG na 24ª–32ª semana de gestação para identificar gestantes com DMG assim como predizer gestantes com risco aumentado de desfechos perinatais adversos.

ANEXOS

Review

Paula B. Renz, Fernando C. Chume, João R.T. Timm, Ana L. Pimentel and Joíza L. Camargo*

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

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Abstract

Background: We conducted a systematic review and meta-analysis to establish the overall accuracy of glycated hemoglobin (HbA_{1c}) in the diagnosis of gestational diabetes mellitus (GDM) diagnosis.
Methods: We searched MEDLINE, EMBASE, SCOPUS and ClinicalTrials.gov up to October 2018, using keywords related to GDM, HbA_{1c} and diagnosis. Studies were included that were carried out with pregnant women without previous diabetes that assessed the performance of HbA_{1c} (index test) compared to the 75 g oral glucose tolerance test (OGTT) (reference test) for the diagnosis of GDM, that measured HbA_{1c} by standardized methods and presented data necessary for drawing 2×2 tables.
Results: This meta-analysis included eight studies, totaling 6406 pregnant women, of those 1044 had GDM. The diagnostic accuracy of HbA_{1c} was reported at different thresholds ranging from 5.4% (36 mmol/mol) to 6.0% (42 mmol/mol), and the area under the curve (AUC) was 0.825 (95% confidence interval [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% (67.5%–92.7%); 24.7% (10.3%–48.5%) and 95.5% (85.7%–98.7%); 10.8% (5.7%–19.41%) and 98.7% (96.2%–99.5%); 12.9% (5.5%–27.5%) and 98.7% (97.6%–99.3%), for the cut-offs of 5.4% (36 mmol/mol), 5.7% (39 mmol/mol), 5.8% (40 mmol/mol) and 6.0% (42 mmol/mol), respectively.
Conclusions: We observed a high heterogeneity among the studies. The effect of ethnicities, different criteria for OGTT interpretation and the individual performance of HbA_{1c} methods may have contributed to this heterogeneity. The HbA_{1c} test presents high specificity but low sensitivity regardless of the threshold used to diagnose GDM. These findings point to the usefulness of HbA_{1c} as a rule-in test. HbA_{1c} should be used in association with other standard diagnostic tests for GDM diagnosis.

Keywords: diagnosis; gestational diabetes; HbA_{1c}; 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 cesarean rates and macrosomia [3]. The detection and adequate treatment of this condition reduces the risks for mothers as well as for babies [3–5].

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 as is used for non-pregnant adults

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using the 2 h 75 g OGTT [2]. The UK National Institute for Health and Care Excellence (NICE) recommendations [9] are based on these criteria; however, they recommended a lower cut-off for fasting glucose. 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 also based on 2 h 75 g OGTT but with lowered thresholds for fasting glucose, 1 h and 2 h glucose. GDM is present if one or more results are altered [10–12]. Since 2013, the WHO has adopted these same IADSPG criteria [2]. According to the ADA, GDM diagnosis can be performed by the one-step 2 h 75 g OGTT using the same threshold diagnostic criteria of IADPSG or the two-step strategy with a 1 h 50 g OGTT screen followed by a 3 h 100 g OGTT for those who screen positive [1].

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

The HbA_{1c} test has been used in clinical practice for monitoring patients with DM since the early 1980s [13], but its use in diagnosis was established only in 2010 [14, 15]. Presently, there are more than 100 certified methods/instruments available for routine HbA_{1c} measurement (http://www.ngsp.org/docs/methods.pdf; accessed 14th December 2018). These methods are mainly based on four principles: immunoassays, ion-exchange chromatography (HPLC), affinity chromatography and enzymatic assays. The International Federation of Clinical Chemistry (IFCC) Working Group on HbA_{1c} Standardization developed a reference system for HbA_{1c} [16] and since the mid 1990s, as well as the National Glycohemoglobin Program (NGSP), work to standardize and align HbA_{1c} methods worldwide [17, 18]. Despite all these international efforts, there are still many situations that may affect HbA_{1c} results, related or not to assay methods, such as the presence of a variant hemoglobin (Hb), anemia and uremia [18, 19]. Recently, the role of race/ethnicity on HbA_{1c} values has been raised [20, 21]. HbA_{1c} values are higher in Blacks, Asians and Latinos when compared to White persons. These factors have limited the use of HbA_{1c} in specific cases.

The cut-off of HbA_{1c} 6.5% (48 mmol/mol) is recommended for DM diagnosis in the general population (Expert Committee 2010), and this cut-off is endorsed by the ADA and WHO [1, 15]. However, its use for the diagnosis of GDM has not been recommended by any current guidelines yet [1, 2, 7, 10]. Results from the HAPO study showed that HbA_{1c} values, like glycemia levels, were significantly associated with all adverse outcomes, and higher levels of maternal HbA_{1c} were related to greater frequency of adverse outcomes [6]. The HbA_{1c} test would be more receptive to this group of patients because of its convenience when compared to the OGTT. However, due to some physiological and analytical factors that might interfere with HbA_{1c} results, it has not yet been included as a diagnostic tool for GDM [1, 18, 19].

During pregnancy, hemoglobin concentrations change overtime, to accommodate the increasing maternal blood volume and the iron needs of the fetus and also there is a decrease in fasting blood glucose levels [2]. Consequently, HbA_{1c} levels are lower in pregnant women than in non-pregnant women. Due to these factors, different reference values are recommended in pregnancy and HbA_{1c} interpretation should consider these factors [22]. In addition, HbA_{1c} is significantly lower in the first trimesters of gestation and HbA_{1c} trimester-specific reference intervals are required throughout pregnancy [23]. HbA_{1c} values vary from 4.0% (20 mmol/mol) to 6.0% (42 mmol/mol) in pregnant women from different populations [24].

Some studies have evaluated the diagnostic accuracy of HbA_{1c} in DMG [25–29]. In a recent meta-analysis with 2812 patients and 5918 controls, which measured HbA_{1c} in pregnant Chinese women, showed that this test is a useful diagnostic tool to confirm GDM [25]. A large cohort study in New Zealand reported that HbA_{1c} ≥5.9% (41 mmol/mol) at the first antenatal visit identified all cases of GDM and was associated with a two-fold risk of congenital anomalies, preeclampsia, shoulder dystocia and a three-fold risk of perinatal deaths [26]. We also showed that HbA_{1c} levels may be a useful diagnostic tool for GDM in pregnant Brazilian women, the HbA_{1c} cut-off point of 5.8% (40 mmol/mol) was able to diagnose 38% of GDM cases by OGTT and 5% of pregnant women classified as GDM negative by the OGTT were identified according to the HbA_{1c} test [27]. Other studies have also highlighted the potential role of HbA_{1c} in the diagnosis and management of GDM [28, 29]. In this study we carried out a systematic review and meta-analysis to determine the diagnostic accuracy of the HbA_{1c} test in the diagnosis of GDM in different populations of pregnant women.

Materials and methods

This meta-analysis is in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement [30] and is in accordance with the Cochrane Handbook for Systematic Reviews

of Diagnostic Test Accuracy [31]. It was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42018041407.

Search strategy and data sources

We searched PubMed (MEDLINE), Embase, SCOPUS and ClinicalTrials.gov, with assistance from our Institution's library professionals, for papers published up to October 2018 using search terms related to GDM, HbA_{1c} 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 those which were not complete were removed and the remaining articles were assessed for eligibility. The revision of titles was followed by reading the abstracts for relevance. Finally, the identification of eligible studies was carried out, based on a full reading of the articles selected by at least two researchers.

Study selection

The inclusion criteria were: (1) cross-sectional or cohort studies that assessed the performance of HbA_{1c} (index test) and 75 g OGTT (reference test) for the diagnosis of GDM; (2) the HbA_{1c} method certified by the National Glycohemoglobin Standardization Program (NGSP; http://www.ngsp.org/, 13 December 2017, date last accessed) and/or the International Federation of Clinical Chemistry (IFCC) [17, 18]; (3) studies that included pregnant women without DM prior to pregnancy or with GDM already diagnosed. Exclusion criteria were: (1) studies that did not perform the 75 g OGTT for the GDM diagnosis; (2) review articles; (3) comments, letters and/or editorials; (4) studies with a language other than English, Spanish or Portuguese; (5) articles published before 1996, as from this date on was when the standardization for the HbA_{1c} methods started [16–18]. Three independent reviewers (PBR, FCC and JRTT) decided which studies were included based upon the eligibility criteria. First, we screened the titles of all papers resulting 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 selected articles qualified for inclusion and performed data extraction from all the included reports. Any disagreements concerning study eligibility or data interpretation were resolved through discussion or, if required, a fourth reviewer was consulted (JLC or ALP).

Data collection and analysis

A data extraction form was developed and the following pieces of 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, HbA_{1c} results); (6) test methods (details of methodology and equipment description for the HbA_{1c} test and the 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 2×2 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 [32]. Disagreements were resolved by consensus or by involving a third reviewer (JLC or ALP). We also evaluated if the articles were presented according to Standards for Reporting of Diagnostic Accuracy (STARD) initiative guidelines [33].

Statistical analysis and data synthesis

We followed the standard methods recommended for diagnostic accuracy meta-analysis studies [34]. For each study, 2×2 contingency tables were constructed with data extracted for TP, TN, FP and FN rates. By a bivariate model using a random effects approach [35] indexes of HbA_{1c} 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 [36]. The DOR is a single indicator that summarizes the diagnostic accuracy of a test, and higher values indicate a better test performance [37]. The overall diagnostic accuracy for the HbA_{1c} test for GDM diagnosis was determined by a summary receiver operating characteristic curve (SROC) for the main cut-off points discussed in each study. Afterwards, hierarchical

summary ROC curves (HSROC) were used to summarize the HbA_{1c} performance for specific cut-offs if four or more studies presented data for the same cut-off [38]. The Fagan nomogram was applied, considering a pre-test probability of 18% for GDM, based on external data from the Metzger et al. study [6], to calculate posttest probabilities for GDM using different HbA_{1c} cut-offs [39]. 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, 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 [40, 41]. We also ran the meta-analyses over again while removing studies one at a time to determine whether a particular study accounted for the heterogeneity. In addition, when data was available, we carried out subgroup analysis by specific cut-off points, HbA_{1c} methods and country of origin. The presence of publication bias was tested by using Deeks' funnel plots [42]. 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 selection and study characteristics

With this strategy 2927 records were identified. Of those, 49 studies were assessed for eligibility. After full-text reading, 40 articles were excluded (one for different language, nine for insufficient data, four for different reference test criterion, three studies performed the diagnostic test in the first gestational trimester and 23 did not meet the research question). Lastly, nine studies met our inclusion criteria [27, 29, 43–49], and of these, only one article [43] was excluded from the meta-analysis due to a lack of relevant information to allow a proper extraction of data as 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 [27, 29, 44–49] (Figure 1).

All studies included in this review totaled 6848 pregnant women, who performed the OGTT and HbA_{1c} test in the second or third trimesters of pregnancy for GDM diagnosis, of those 1128 were diagnosed with GDM (15.2%). Table 1 summarizes the characteristics of all selected studies. Three studies had a prospective design [43, 47, 48], one was a retrospective study [49] and five were cross-sectional studies [27, 29, 44–46]. All studies were written in English and published between 2005 and 2017. Four studies were from India, while the 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 [44] presented a high risk of bias in the patient selection, flow and timing; in this study 1459 pregnant women participated, 33 of which were in the first trimester of pregnancy while the remaining women were in the second trimester of pregnancy. Another study [43] 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 [27] presented an unclear risk of bias in flow and timing, as 120 pregnant were diagnosed using the WHO 1999 diagnostic criteria and 142 were diagnosed using the IADPSG criteria. Only one article followed the recommendations and was presented according to the STARD guidelines [27].

Meta-analysis

Overall diagnostic accuracy

For this analysis we considered the main HbA_{1c} cut-offs discussed in each article [27, 29, 44–49]. HbA_{1c} thresholds ranged from 5.4% (36 mmol/mol) to 6.0% (42 mmol/mol). A total of 6406 pregnant women were included in this analysis, of those, 1044 were diagnosed with GDM. Using data from these eight studies, DOR was 6.97

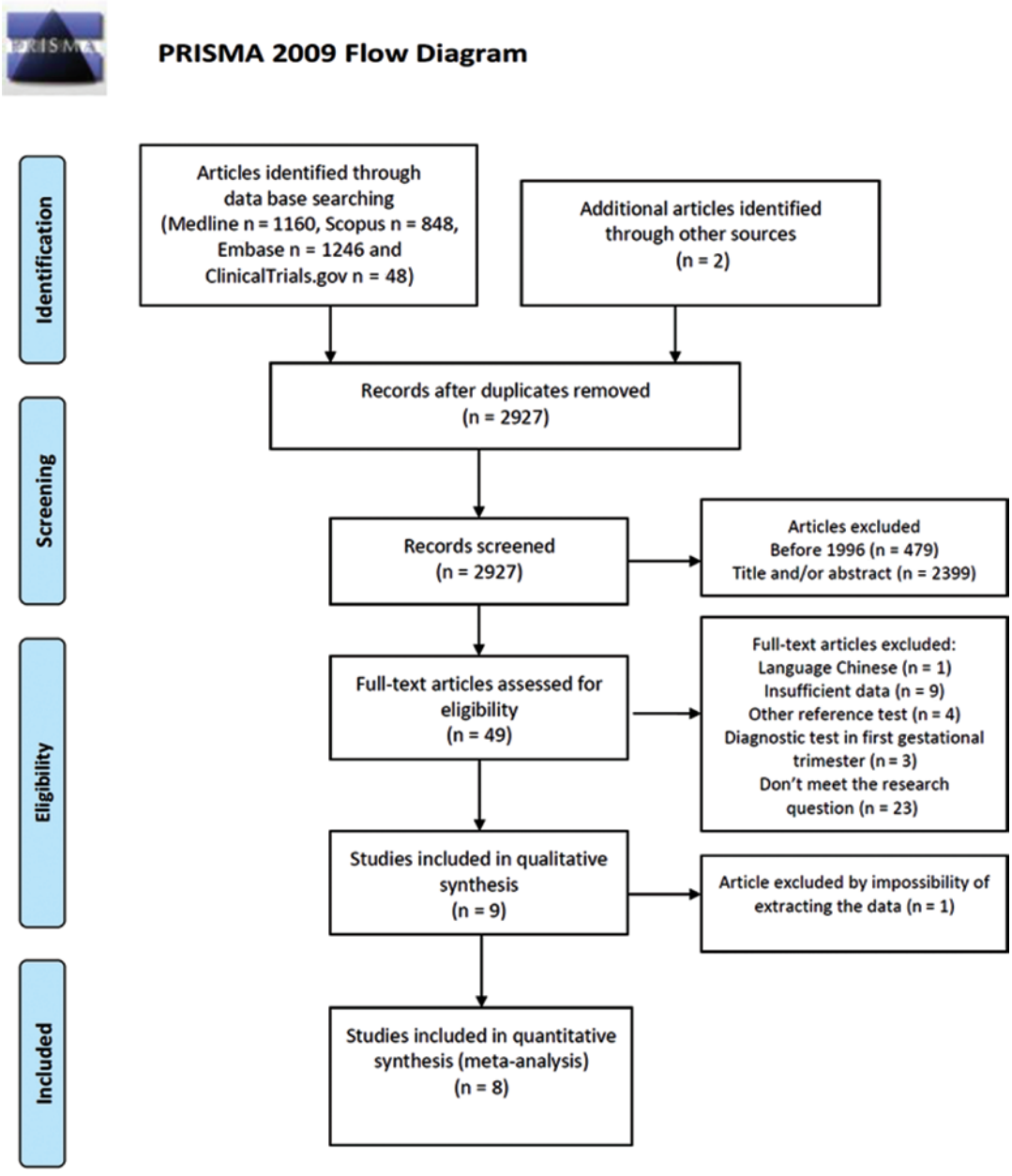

Figure 1: Flowchart of the article selection process (from [30]).

(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 the HbA_{1c} threshold on diagnostic accuracy

The forest plot in Figure 3 shows the sensitivity and specificity of HbA_{1c} for the detection of GDM across all eight included studies. For studies reporting accuracy at more than one threshold, 2×2 tables were built for each cut-off. The cut-offs 5.4% (36 mmol/mol), 5.7% (39 mmol/mol), 5.8% (40 mmol/mol) and 6.0% (42 mmol/mol) were reported by at least four studies and their data were included in the forest plots. Table 3 summarizes the accuracy measures for these cut-offs.

HbA_{1c} ≥5.4% (36 mmol/mol) for the diagnosis of GDM

Four studies evaluated the cut-off of 5.4% (36 mmol/mol) [27, 29, 44, 45], totaling 2808 pregnant women. The HSROC curve showed an AUC of 0.779 (95% CI

Table 1: Characteristics of selected studies.

Reference	Country	Study design	Age, years	Diagnostic criteria, 75 g OGTT	HbA_{1c}, %	GDM incidence, %	Pregnancy, weeks	HbA_{1c} cut-off, %[c]	Total, n	HbA_{1c} method
Agarwal 2005	Arab 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[a]	India	Cross-sectional	26.1 ± 3.9	IADPSG	Without GDM 4.9 ± 0.5 With GDM 5.2 ± 0.5	13	12–26	5.4/**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**/5.7/5.8/6.0	480	Imunoassay by DCA 2000 Siemens
Rajput 2012	India	Cross-sectional	16–30	ADA/IADPSG	With GDM 5.73 ± 0.34	7 by ADA 24 by IADPSG	24–28	**5.95**/5.45	607	Immunoassay Conelab30i
Renz 2015[b]	Brazil	Cross-sectional	23–35	WHO and/or IADPSG	Without GDM 5.1 ± 0.4 With GDM 5.5 ± 0.5	33	22–32	5.3/5.7/**5.8**/6.0	262	HPLC (Variant II Turbo analyzer) Biorad Laboratories
Saxena 2017	India	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	India	Prospective	No GDM 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**/5.7/5.8	1959	HPLC (Variant II Turbo analyzer) Biorad Laboratories

Data are expressed as mean ± SD or range. [a]Thirty-three pregnant in 1st gestational trimester; [b]120 pregnant by the WHO 1999 diagnostic criteria; [c]principal HbA_{1c} 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.

Study	Checklist QUADAS-2						
	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.

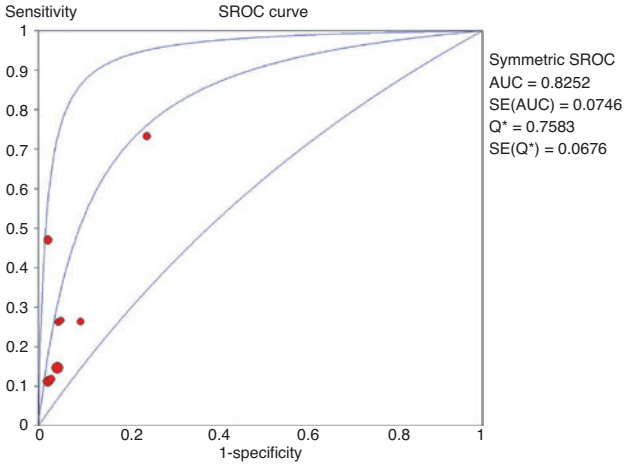

Figure 2: Summary receiver operating characteristic curves (SROC) of HbA_{1c} for all eight studies.

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%–92.7%) (Table 3). After re-running the meta-analysis by removing one paper at a time, when removing the study by Bhavadharini et al. [44], no DOR heterogeneity was found ($I^2 = 0\%$), pooled sensitivity decreased and the pooled specificity was the same (39% [95% CI 33%–44%] and 83% [95% CI 81%–84%]), respectively. However, after carefully reviewing this study, we were unable to explain the reasons why it contributed to the increase in heterogeneity for this cut-off and the results from the primary meta-analysis were considered.

HbA_{1c} ≥5.7 (39 mmol/mol) for the diagnosis of GDM

Five studies presented data for cut-off of 5.7% (39 mmol/mol) [27, 45, 47–49], totaling 3540 pregnant women. The 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

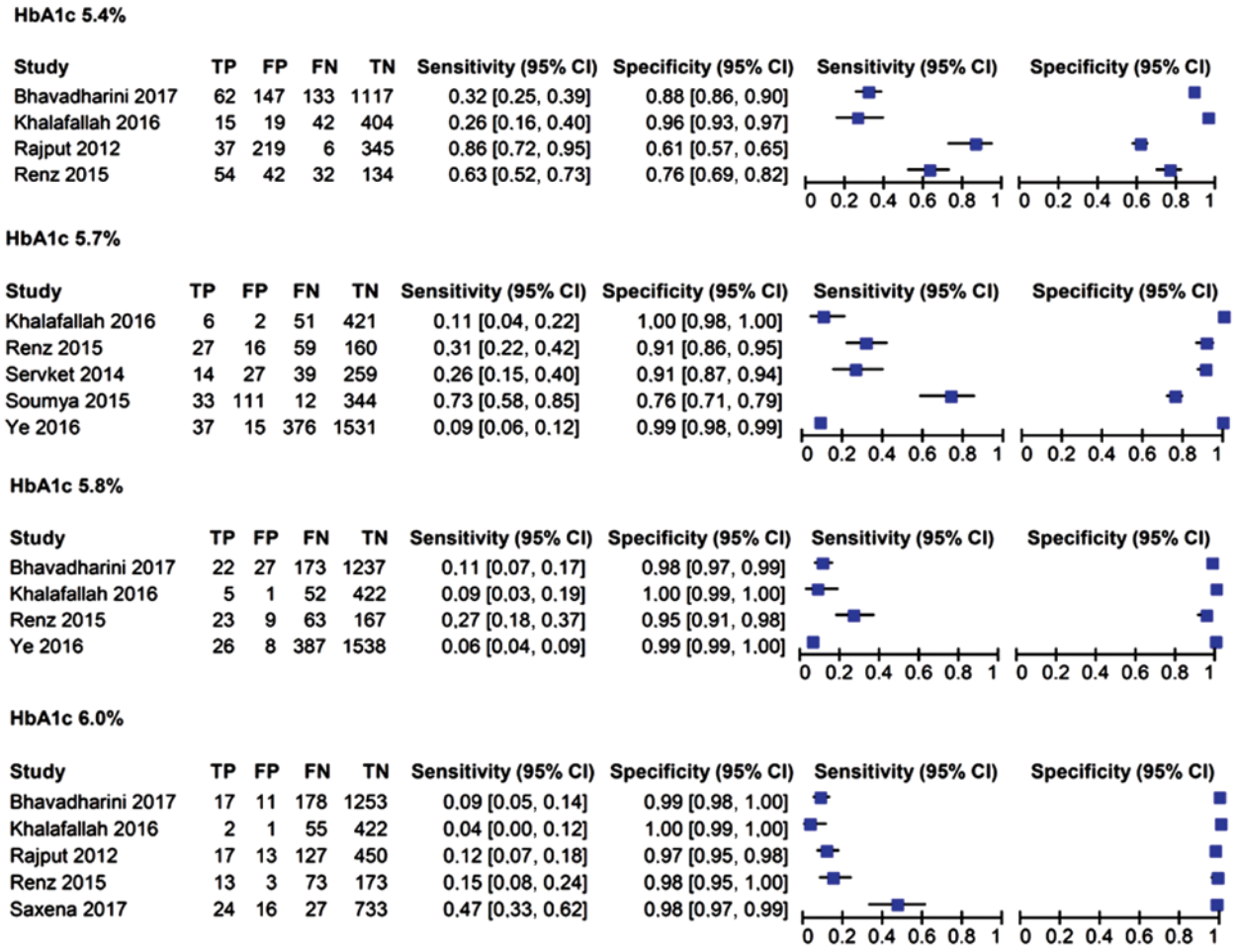

Figure 3: Forest plot of the sensitivity and specificity of HbA_{1c} cut-offs in the diagnosis of GDM.
TP, true positive; FP, false positive; FN, false negative; TN, true negative. The blue square depicts the sensitivity and specificity for each study and the horizontal line represents the corresponding 95% confidence interval for these estimates.

the pooled specificity was 95.5% (95% CI 85.7%–98.7%) (Table 3). After re-running the meta-analysis by removing one paper at a time, no article explained the moderate DOR heterogeneity for this cut-off and we were unable to explain the reasons for this heterogeneity.

HbA_{1c} ≥5.8% (40 mmol/mol) for the diagnosis of GDM

Four studies evaluated the threshold of 5.8% (40 mmol/mol) [27, 44, 45, 49], totaling 4160 pregnant women. The 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%–99.5%) (Table 3). This meta-analysis showed low heterogeneity thus sensitive analysis was not carried out.

HbA_{1c} ≥6.0% (42 mmol/mol) for the diagnosis of GDM

Five studies reported data at the threshold of 6.0% (42 mmol/mol) [27, 29, 44–46], totaling 3608 pregnant women. The 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%–99.3%) (Table 3). After re-running the meta-analysis by removing one paper at a time, by removing the Saxena et al. [46] study, 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 of the IADPSG criteria, this fact could explain the DOR heterogeneity in this subgroup meta-analysis. However, pooled sensitivity and pooled specificity for HbA_{1c} ≥6.0% (42 mmol/mol) after excluding

Table 3: Summary of findings.

Review question: What is the diagnostic accuracy of HbA_{1c} 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 2×2 data.
Index test: HbA_{1c}
Reference standard: 75 g OGTT

Subgroup HbA_{1c}, % [mmomol]	Studies (n)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	AUC	Interpretation for every 1000 pregnant women tested prevalence = 18%
5.4 [36]	4	0.503 (0.25–0.76)	0.837 (0.67–0.93)	0.779	91 cases of GDM will be detected, 89 cases will be missed, and there will be 134 false GDM diagnoses
5.7 [39]	5	0.247 (0.10–0.48)	0.955 (0.86–0.99)	0.741	44 cases of GDM will be detected, 136 cases will be missed, and there will be 37 false GDM diagnoses
5.8 [40]	4	0.107 (0.06–0.19)	0.987 (0.96–0.99)	0.626	19 cases of GDM will be detected, 161 cases will be missed, and there will be only 11 false GDM diagnoses
6.0 [42]	5	0.129 (0.05–0.27)	0.987 (0.98–0.99)	0.927	23 cases of GDM will be detected, 157 cases will be missed, and there will be 11 false GDM diagnoses

AUC, area under the curve; CI, confidence interval.

this study were practically unchanged and were 10.2% (95% CI 7.6%–13.2%) and 98.8% (95% CI 98.3%–99.2%), respectively.

Effect of other variables on diagnostic accuracy

We also investigated the effect of different methods of HbA_{1c} measurement and the country of origin of patients to explain the variability among studies. For this analysis, we considered the main HbA_{1c} cut-offs discussed in each article. Four studies used HPLC [27, 44, 48, 49] and four used immunoassays [29, 45–47] to measure HbA_{1c}. We observed low variability when we pooled studies with HbA_{1c} 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. [46] 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 [29, 44, 46–49] (DOR = 4.77 [95% CI 3.55–6.40; I^2 = 38.4%]) and absent when we evaluated non-Asian studies [27, 45] (DOR =7.21 [95% CI 4.15–12.54; I^2 = 0.0%]). There was no data available to investigate the effect of anemia, iron supplementation and presence of variants hemoglobin on results heterogeneity.

Publication bias

Although investigation of reporting bias in diagnostic accuracy data is not well established, we used the method of Deeks [42], that appears to be most appropriate, which indicated that there was no potential publication bias (p = 0.112).

Post-test probabilities

Considering the pre-test probability of 18% for GDM and the PLR and NLR for cut-offs 5.4% (36 mmol/mol), 5.7% (39 mmol/mol), 5.8% (40 mmol/mol) and 6.0% (42 mmol/mol) we calculated the post-test probabilities for GDM applying the Fagan's nomogram (Figure 5). The post-test probabilities were 40% and 12% for HbA_{1c} ≥5.4% (36 mmol/mol) and <5.4% (36 mmol/mol); 55% and 15% for HbA_{1c} ≥5.7% (39 mmol/mol) and <5.7% (39 mmol/mol); 64% and 17% for HbA_{1c} ≥5.8% (40 mmol/

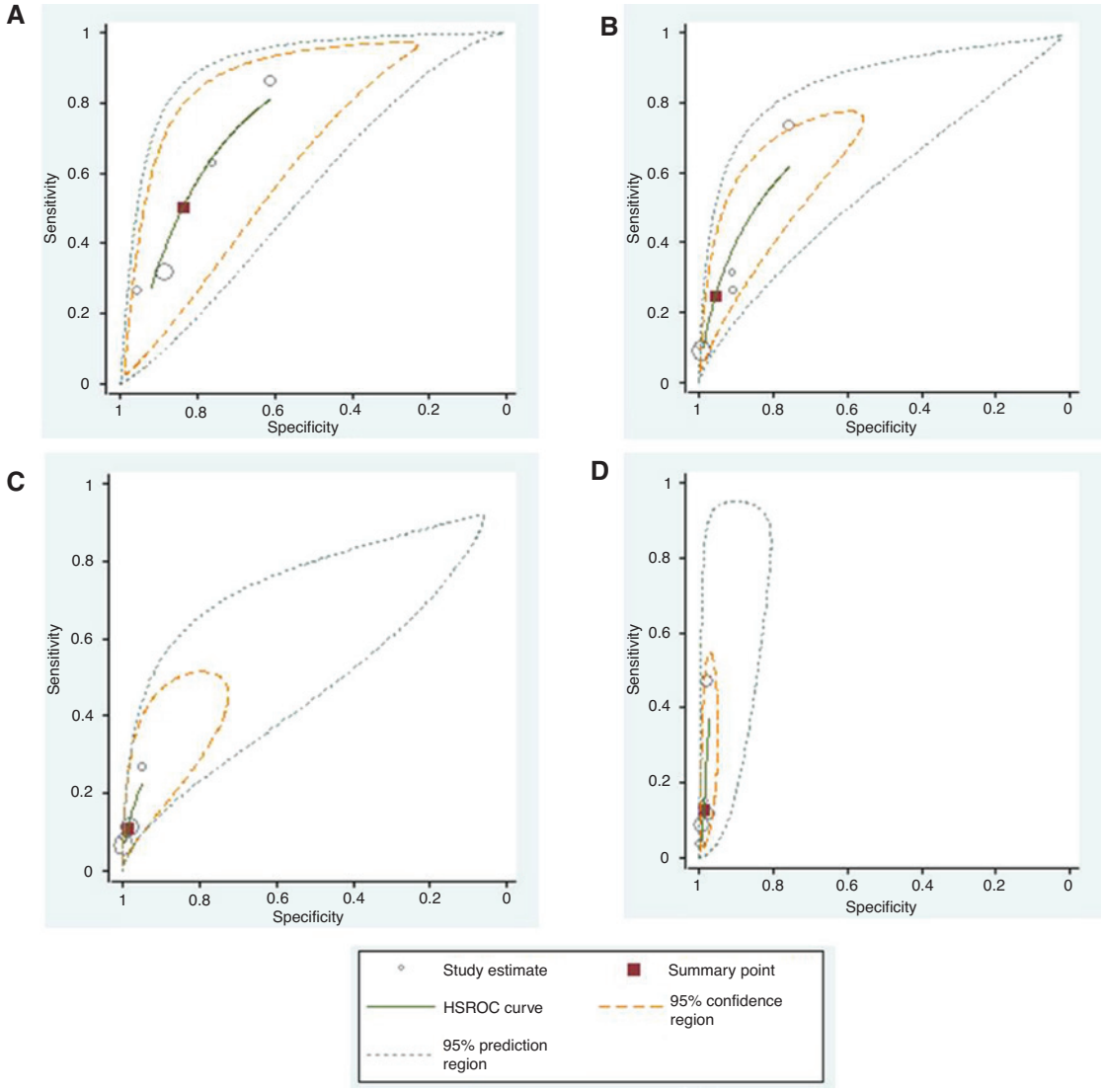

Figure 4: Hierarchical summary receiver operating characteristic curves of HbA_{1c} at different cut-off points for GDM. (A) 5.4% (36 mmol/mol); (B) 5.7% (39 mmol/mol); (C) 5.8% (40 mmol/mol) and (D) 6.0% (42 mmol/mol).

mol) and <5.8% (40 mmol/mol); 69% and 16% for HbA_{1c} ≥6.0%(42 mmol/mol) and <6.0% (42 mmol/mol); respectively.

Discussion

Summary of the main results

In this meta-analysis we included eight studies, covering 6406 pregnant women, and of those 1044 were diagnosed with GDM. The diagnostic accuracy of the HbA_{1c} test was reported at different thresholds ranging from 5.4% (36 mmol/mol) to 6.0% (42 mmol/mol). The 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 the HbA_{1c} test. Four studies evaluated the cut-off of 5.4% (36 mmol/mol) [27, 29, 44, 45], totaling 2808 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%–92.7%), respectively. For a cut-off of 5.7% (39 mmol/mol), five studies presented data [27, 45, 47–49], totaling 3540 pregnant women. The pooled sensitivity and specificity for these studies was 24.7% (95% CI 10.3%–48.5%) and 95.5% (95% CI 85.7%–98.7%), respectively. Four studies

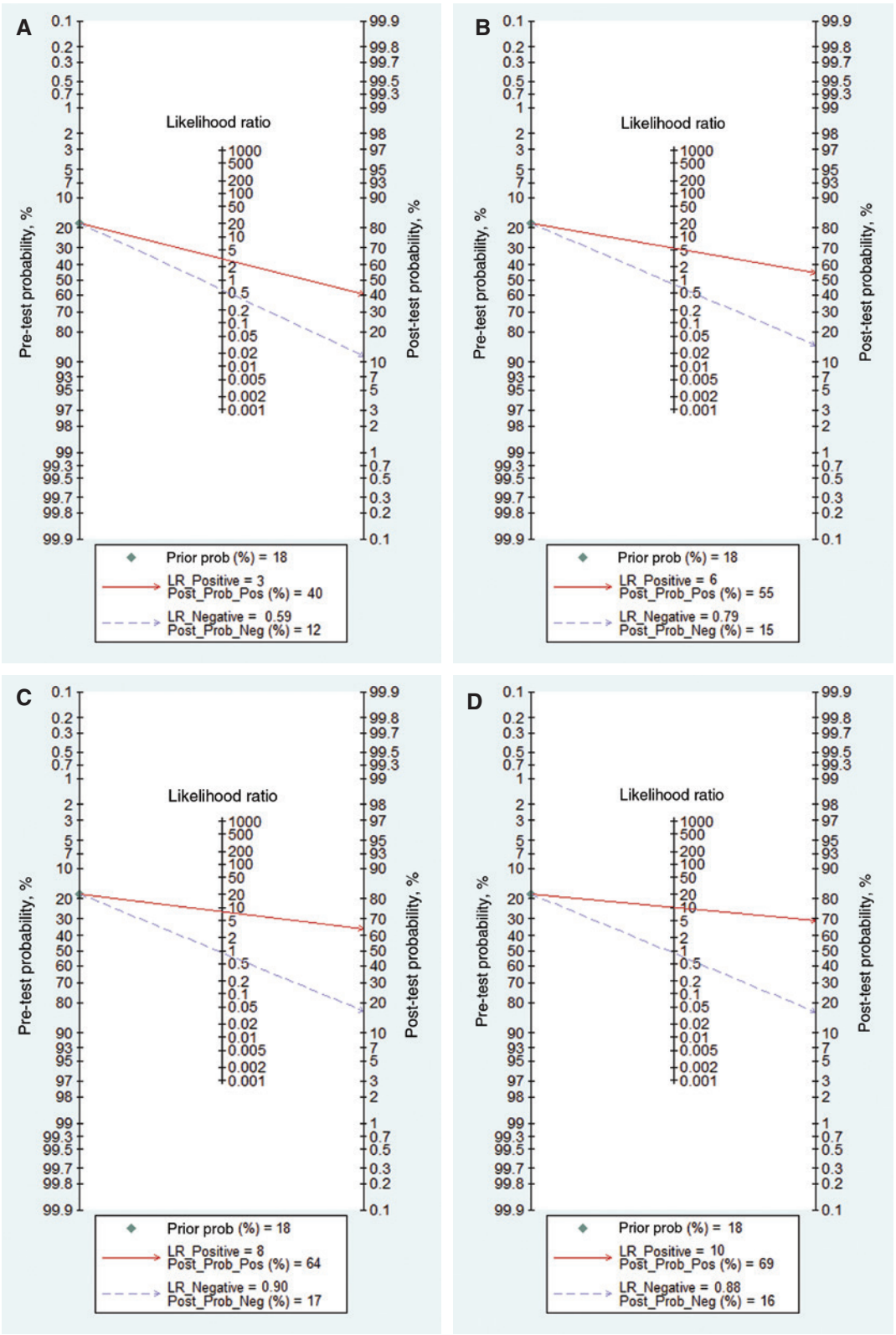

Figure 5: Fagan's nomograms for HbA_{1c} test, showing post-test probabilities for GDM.
(A) HbA_{1c} ≥5.4% (36 mmol/mol); (B) 5.7% (39 mmol/mol); (C) 5.8% (40 mmol/mol) and (D) 6.0% (42 mmol/mol).

evaluated the threshold of 5.8% (40 mmol/mol) [27, 44, 45, 49], totaling 4160 pregnant women yielding a pooled sensitivity and specificity of 10.8% (95% CI 5.7%–19.41%) and 98.7% (95% CI 96.2%–99.5%). Five studies reported data for the threshold of 6.0% (42 mmol/mol) [27, 29, 44–46], totaling 3608 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%–99.3%), respectively.

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 the HbA_{1c} test in the diagnosis of GDM. A recent study in pregnant Chinese women [25] that aimed to establish the overall accuracy of the HbA_{1c} test for the diagnosis of patients with GDM, after a systematic review, included 5918 controls and 2812 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 a high level of overall accuracy for the HbA_{1c} test in the diagnosis of GDM.

In a prospective study that enrolled 1989 pregnant Taiwanese women [50], the AUC was 0.70 and the optimal HbA_{1c} cut-off point to predict GDM was 5.7% (39 mmol/mol) (sensitivity = 45.2% and specificity = 84.1%). However, the reference test adopted in this study was two-step OGTT recommended by National Health Institute (NHI). The results are in agreement with this review, showing low sensitivity and relative high specificity for HbA_{1c} to diagnose GDM. Additionally, the study by Li et al. [51] reported a positive correlation of HbA_{1c} with blood glucose in pregnancy affected by GDM. They showed an AUC for HbA_{1c} of 0.854 ($p < 0.01$). When HbA_{1c} was 5.43% (36 mmol/mol), sensitivity and specificity were 0.832 and 0.764, respectively. Hanna et al. [52] examined the concordance between different criteria for GDM diagnosis and observed an increased proportion of women with an HbA_{1c} ≥6.0% (42 mmol/mol) in the discordant cases. They then evaluated the performance of this HbA_{1c} threshold in the diagnosis of GDM and found a similar sensitivity and specificity of HbA_{1c}, around 22% and 97%, respectively, irrespective of the criteria used to diagnose GDM. They concluded that the HbA_{1c} 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. [53] evaluated through a systematic review a different test strategy for the diagnosis of GDM and concluded that there is insufficient evidence to suggest which strategy is best for diagnosing GDM, although HbA_{1c} 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 a low risk of bias and applicability concerns. As limitations for this study, we highlight: First, although we only included studies that measured HbA_{1c} 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 HbA_{1c} sensitivity. Despite our efforts to analyze and exlpain the heterogeneity among studies, scarcity of data regarding interferent factors, such as anemia, iron supplementation and the presence of variants of haemoglobin in the original papers limited our analyses. However, we were able to draw attention to the likely effect of ethnicity and the use of different criteria for OGTT interpretation. One study used the WHO 1999 criteria [44] and after its exclusion a low heterogeneity was observed. Heterogeneity was also low when we pooled only studies from Asia [29, 44, 46–49] and absent when we evaluated non-Asian studies [27, 45], pointing to the effect of ethnicity on HbA_{1c} values in different populations [20, 21]. All these possible interferences might have affected in different ways the HbA_{1c} levels measured by the method used in the primary studies. Third, only one article [27] followed the recommendations and was presented according to the STARD guidelines [33] which may have affected the quality of reporting of the other studies.

Applicability of findings to the review question

To make sense of the results of the meta-analysis and to assess the false-error rates, we calculated the post-test probabilities for GDM applying the Fagan's nomogram, we considered the test performance estimates based on external data from the Metzger et al. study [6], with a pre-test probability of 18% for GDM and the PLR and NLR

for cut-offs 5.4% (36 mmol/mol), 5.7% (39 mmol/mol), 5.8% (40 mmol/mol) and 6.0% (42 mmol/mol). The post-test probabilities for a positive test were 40%, 55%, 64% and 69% for HbA_{1c} ≥5.4% (36 mmol/mol), HbA_{1c} ≥5.7% (39 mmol/mol), HbA_{1c} ≥5.8% (40 mmol/mol) and HbA_{1c} ≥6.0% (42 mmol/mol); respectively. The post-test probabilities for a negative test for these cut-offs were low and ranged from 12 to 17%, like the pre-test probability of 18%. HbA_{1c} results ≥5.4% (36 mmol/mol) increase at least two-fold the probabilitiy for GDM whereas HbA_{1c} results <5.4% (36 mmol/mol) do not alter the initial probability of GDM.

Conclusions

Limited evidence provided by the studies included in this review suggests that HbA_{1c} tests, regardless of the threshold used to diagnose GDM, result in few false-positive GDM cases but very high levels of false negative GDM cases, with a high level of specificity across all the population groups described here. These findings point to the usefulness of HbA_{1c} cut-offs of 5.7% (39 mmol/mol), 5.8 (40 mmol/mol) or 6.0% (42 mmol/mol) as rule-in tests for the diagnosis of GDM. However, it means that irrespective of the cut-off adopted, a negative result will require further investigation through a more sensitive test for confirmation of the diagnosis. The prognostic value of HbA_{1c} for GDM adverse outcomes needs further evaluation by prospective studies and it is beyond the scope of this review.

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Registration number: PROSPERO registration-CRD42018 041407.

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ORIGINAL ARTICLE

Is there a role for glycated albumin in the diagnosis of gestational diabetes mellitus?

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Abstract

Background Studies in the general population have advocated glycated albumin (GA) as a useful alternative to glycated haemoglobin (HbA1c) under conditions wherein the latter does not reflect glycaemic status accurately. There are few studies in other populations, especially in pregnant women. Therefore, the aim of this study was to assess the clinical utility of GA in the diagnosis of gestational diabetes mellitus (GDM).

Materials and methods This diagnostic test accuracy study was performed in 149 Brazilian women at 24–28 weeks of gestation referred for an oral glucose tolerance test (OGTT) in a tertiary university hospital. Receiver Operating Characteristic (ROC) curves were used to access the performance of GA and HbA1c in the diagnosis of GDM by the reference OGTT.

Results GDM by OGTT (IADPSG criteria) was detected in 18.8% of participants. According to ROC analysis, the area under the curve (AUC) for GA was 0.531 (95% CI: 0.405–0.658, $p = 0.065$) lower than that for HbA1c [0.743 (95% CI: 0.636–0.849; $p \leq 0.001$] for the detection of GDM ($p = 0.004$). The equilibrium cut-off value for GA was 12.6%; sensitivity and specificity in this cut-off point were 53.6% and 54.2%, respectively.

Conclusions GA at 24–28 weeks of gestation does not have ability to correctly discriminate those with and without GDM. In summary, the lack of sensitivity found in our results do not support the solely use of GA in the diagnosis of GDM.

Keywords Gestational diabetes mellitus · Glycated albumin · HbA1c · Oral glucose tolerance test · Diagnostic accuracy

Highlights

- Previously studies in general population reported that glycated albumin (GA) presents similar accuracy as HbA1c for detecting diabetes.
- The present study showed that in pregnant women the area under the curve (AUC) for GA in the diagnosis of gestational diabetes mellitus (GDM) was much lower than AUC for HbA1c.
- Unlike HbA1c, GA does not have the ability to correctly discriminate those with and without GDM.
- Our findings may provide a cautious approach when assessing glycaemic status using GA in pregnant women, since pregnancy can be considered a confounding factor.

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Abbreviations

GDM	gestational diabetes mellitus
FPG	fasting plasma glucose
OGTT	oral glucose tolerance test
1h-PG	1-h plasma glucose after a 75-g
OGTT	2h-PG - 2-h plasma glucose after a 75-g
OGTT	HbA1c glycated haemoglobin
GA	glycated albumin
GSP	glycated serum proteins
ROC	receiver operating characteristic
AUC	area under the ROC curve
SD	standard deviation
LR	likelihood ratio
WHO	World Health Organization
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
STARD	Standard for Reporting Diagnostic Accuracy
HCPA	Hospital de Clinicas de Porto Alegre

Introduction

Diabetes that is first diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation is named gestational diabetes mellitus (GDM) [1]. This condition carries adverse effects for the mother and neonate. The detection and treatment of this condition may reduce the risk of adverse maternal, foetal and neonatal outcomes [2–4]. Tough GDM may be asymptomatic and many people do not have the classic diabetes risk factors. Therefore, the World Health Organization (WHO) recommends the screening of GDM for all pregnant women [5].

The Hyperglycaemia and Adverse Pregnancy Outcome study, a large-scale multinational cohort study, demonstrated that risk of adverse maternal, foetal and neonatal outcomes continuously increased with maternal glycemia at 24–28 weeks of gestation, even within ranges previously considered normal for pregnancy [6]. These results support the need for screening for GDM between the 24th and 28th week of gestation [1]. However, the lack of threshold for risk in most complications led to great controversy about the diagnostic criteria for GDM. Different diagnostic criteria will identify different degrees of maternal hyperglycaemia and maternal/foetal/neonatal risk, leading to conflicted recommendations from experts on optimal strategies for the diagnosis of GDM [1]. GDM diagnosis may be based in one-step 75-g OGTT [7] or two-step approach with a 50-g OGTT screening followed by a 100-g OGTT for those who screened positive [8]. The Brazilian Diabetes Society [9] recommends International Association of the Diabetes and Pregnancy Study Groups (IADPSG) strategy which is also adopted by WHO.

Of late, HbA1c, the current standard test for monitoring glycaemic control, is also considered as diagnostic tool for diabetes in the general population [1]. Recent studies examine the validity of HbA1C in different population, including among pregnant women. A systematic review and meta-analysis that examined the overall accuracy of HbA1c in the diagnosis of GDM showed that HbA1c presented high specificity but low sensitivity regardless of the threshold used to diagnose GDM [10]. HbA1c performance in pregnant women is similar to the one reported to HbA1c when it is used as diagnostic tool for diabetes in general population [1]. Although advantages of HbA1c over glucose-based tests includes patient's comfort (fasting not required) and measurement stability, there are some situations that HbA1c is not suitable to use like conditions with altered blood red cell turnover, such as anaemia [11], a common condition in pregnant women. Therefore, it is important to consider alternative procedures for the diagnosis of GDM.

Glycated albumin (GA) is a test that has gained prominence as an alternative glycaemic marker [12]. GA is a measure of glycaemia based on the amount of glucose in serum or plasma attached to albumin, rather than to haemoglobin. The assay is well standardised and has been automated for high throughput analysis. GA reflects short-term mean glycaemia (2–3 weeks), rather than 2–3 months mean glycaemia observed for HbA1c [12]. Like HbA1c, GA correlates well with diabetic complications, and even death in people with diabetes [13, 14].

In addition, GA is haemoglobin/erythrocyte independent and the performance of the test in the diagnosis of diabetes is similar to HbA1c in most studies [15–20]. Therefore, those studies advocate GA as a useful alternative to HbA1c under conditions wherein the latter does not reflect glycaemic status accurately.

Although evidences about GA performance in diagnosis and screening of diabetes have been available in general populations, few studies exist in other populations, especially in pregnant women [21, 22].

Then, the current study was designed to assess the clinical utility of GA in the diagnosis of GDM.

Materials and methods

This cross-sectional study of diagnostic accuracy was designed and reported according to Standard for Reporting Diagnostic Accuracy (STARD) initiative guidelines [23].

Participants

This study includes pregnant women in 24–28 weeks of gestation that were referred to perform OGTT in Hospital de

Clinicas de Porto Alegre (HCPA) between September 2009 and July 2012. All participants were previously included in a study evaluating the use of HbA1c for the diagnosis of GDM. During this previous study they signed an informed consent term and provided clearance for the use of stored material and data in related future studies [24]. The present study protocol was approved by the Ethics Committee of the Hospital de Clinicas de Porto Alegre (GPPG-HCPA) and is registered by the number GPPG 2018–0409.

Exclusion criteria for this study were pregnant women under 18 years old, presence of twin pregnancy, women with established diagnosis of diabetes or who were receiving anti-diabetic medication, presence of clinical conditions known to interfere or lead to misinterpretation of GA and/or HbA1c results, such as albumin levels <3.0 g/dl, severe anaemia (haemoglobin <7 g/dL), presence of variant haemoglobin, recent transfusion, rheumatic disorder, hepatic cirrhosis, nephrotic syndrome, chronic kidney disease, untreated thyroid dysfunction and/or Cushing syndrome [11, 12].

Glycaemic status was defined according to recommendations of American Diabetes Association using one step 75 g OGTT strategy—IADPSG criteria [1]. GDM was defined by: (a) fasting plasma glucose (FPG) ≥ 92 mg/dL and/or *(b)* 1 h plasma glucose after ingestion of 75 g of glucose (1h-PG) ≥ 180 mg/dL and/or (c) 2 h plasma glucose after ingestion of 75 g of glucose (2h-PG) ≥ 180 mg/dL and/or 2 h ≥ 153 mg/dL.

Laboratory analysis

All pregnant women underwent a standard 75 g OGTT after an overnight fast of at least 8 h. Blood samples for glucose determination were collected by venipuncture into tubes containing sodium fluoride at fasting, 1- and 2-h after 75 g glucose oral intake. Plasma glucose concentrations were measured by colorimetric enzymatic method in the biochemistry automated analyser Cobas® c702 (Roche Diagnostics, Germany).

HbA1c were measured in K2EDTA-anticoagulated whole blood by high performance liquid chromatography (HPLC) using VARIANT II™ System (BioRad Laboratories, Hercules, CA, USA). This HbA1c assay is certified by the National Glycohemoglobin Standardization Program and aligned to the DCCT reference and the International Federation of Clinical Chemistry reference [11]. The inter-assay coefficient of variation (CV) for HbA1c method was <3.0%.

Fasting serum samples were stored at −80 °C until they were used for measurement of GA. GA was determined by an enzymatic method (GlycoGap®, Diazyme Laboratories, Poway, CA) in the automated analyser Cobas® c702 (Roche Diagnostics, Germany), previously validated in our laboratory [25] and the inter-assay CV for this assay was 3.0%. Total albumin was measured with bromocresol green colorimetric method. GlycoGap® GA assay quantifies the total of glycated serum proteins (GSP, μmol/L), which are converted to percent of GA by the following conversion equation: GA (%) = {[GSP (μmol/L) × 0.182 + 1.97]/total albumin (g/dL)} + 2.9 [25].

Statistical analysis

Sample size was calculated based on the results obtained in studies in the general population, where GA has accuracy similar to that of HbA1c [15–20]. Considering a significance level of 5%, power of 80% and an area on the expected curve of 0.714, as found in previous study during evaluation of the performance of the HbA1c test to detect GDM [24], the total sample size of 54 individuals was reached, 27 in each group. Adding 10% for possible losses and refusals, the sample size would require 60 participants. Sample size calculations were carried out in PSS Health tool online version [26].

Data are expressed as mean ± standard deviation (SD) or frequencies (%). Data normality were examined using histograms and Shapiro–Wilk test. Student's *t*-tests and chi-squared were used as appropriate. Pearson's correlation coefficients were calculated to assess correlations between GA and FPG, 1h-PG, 2h-PG and HbA1c. Receiver Operating Characteristic (ROC) curve was used to analyse the performance of the HbA1c test to diagnose GDM considering the OGTT as reference diagnostic criteria. All areas under the curves (AUC) were pairwise compared by DeLong's test.

The IBM SPSS software for Windows, version 20.0 (Statistical Package for Social Sciences—Professional Statistics, IBM Corp, Armonk, USA) and MedCalc, version 19.1 (MedCalc software, Ostend, Belgium) were used for data analysis. P values < 0.05 were considered significant.

Results

A total of 149 pregnant women between 24 and 28 weeks of gestation and without pre-existing diabetes were included in this study. Twenty-eight (18.8%) participants were diagnosed with GDM using OGTT as diagnostic criteria. The characteristics of these participants are shown in Table 1. Participants with GDM were older and had higher values of FPG, 1hPG, 2hPG and HbA1c. No significant difference was detected in GA levels between the two groups.

GA significantly correlated with HbA1c only on pregnant women with GDM (women with GDM: $r = 0.405$, $p = 0.033$ and women without DM: $r = -0.081$, $p = 0.379$). Whereas GA did not correlate significantly with FPG, 1hPG

Table 1 Clinical and laboratory characteristics of participants in the study

Characteristics	All participants ($n = 149$)	Without GDM ($n = 121$)	With GDM ($n = 28$)	p value
Age (years)	28.5 ± 6.6	27.9 ± 6.7	30.7 ± 5.8	<0.05
Gestational age (weeks)	26.5 ± 4.6	26.7 ± 4.2	25.6 ± 6.3	0.239
Systolic BP (mmHg)	113.7 ± 12.9	105.3 ± 29.4	114.6 ± 30.1	0.185
Diastolic BP (mmHg)	70.7 ± 11.2	71.4 ± 13.3	76.4 ± 12.0	0.115
HbA1c [mmol/mol; (%)]	32 ± 4.4 (5.1 ± 0.4)	32 ± 3.3 (5.1 ± 0.3)	36 ± 4.4 (5.4 ± 0.4)	<0.05
Glycated albumin (%)	12.7 ± 1.2	12.7 ± 1.2	12.7 ± 1.5	0.852
FPG (mg/dL)	81.4 ± 8.4	78.9 ± 5.6	92.2 ± 9.9	<0.05
1 hPG (mg/dL)	130.3 ± 35.2	119.1 ± 25.6	180.2 ± 28.1	<0.05
2 hPG (mg/dL)	114.9 ± 28.1	127.4 ± 36.4	150.39 ± 29.5	<0.05
Serum albumin (g/l)	3.7 ± 1.3	3.7 ± 0.5	3.8 ± 0.4	0.236
Haemoglobin (g/l)	11.7 ± 0.9	11.7 ± 0.9	12.1 ± 0.8	<0.05
Haematocrit (%)	34.7 ± 2.8	34.4 ± 2.8	35.7 ± 2.4	<0.05

Data are expressed as mean ± SD or frequencies. GDM was defined as FPG ≥ 92 mg/dL and/or 1hPG ≥ 180 mg/dL and/or 2hPG ≥ 153 mg/dL after an oral glucose tolerance test

BP blood pressure, *GDM* gestational diabetes mellitus, *FPG* fasting plasma glucose, *HbA1c* glycated haemoglobin, *1hPG* plasma glucose 1 h after oral glucose, *2hPG* plasma glucose 2 h after oral glucose

Table 2 Correlations of GA, HbA1c, FPG and 2hPG by GDM status

(A) Pregnant women without GDM ($n = 121$)					
	GA	FPG	1hPG	2hPG	HbA1c
GA	1	−0.012	−0.089	−0.017	−0.081
FPG		1	0.194[a]	0.275[b]	0.294[b]
1hPG			1	0.045	0.162
2hPG				1	0.279[b]
HbA1c					1
(B) Pregnant women with GDM ($n = 28$)					
	GA	FPG	1hPG	2hPG	HbA1c
GA	1	0.209	−0.049	0.069	0.405[a]
FPG		1	0.124	−0.146	0.378[a]
1hPG			1	0.053	−0.390[a]
2hPG				1	0.209
HbA1c					1

FPG fasting plasma glucose, *1hPG* plasma glucose 1 h after oral glucose, *2hPG* plasma glucose 2 h after oral glucose, *HbA1c* glycated haemoglobin, *GA* glycated albumin, *GDM* gestational diabetes mellitus

[a]Correlation is significant at the 0.01 level (two-tailed)

[b]Correlation is significant at the 0.05 level (two-tailed)

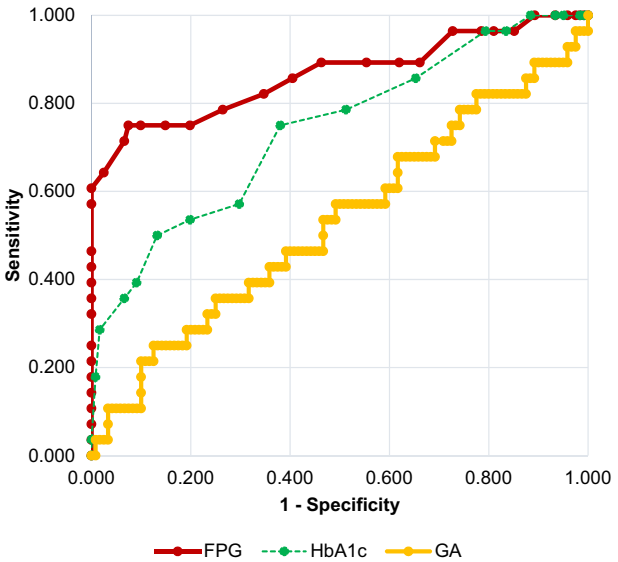

Fig. 1 Receiver operating characteristic (ROC) curves to access the performance of FPG, GA and HbA1c in the diagnosis of GDM by OGTT. The AUC value for FPG was 0.865 (SE: 0.048, 95% CI: 0.772–0.958, $p < 0.001$), GA was 0.531 (SE: 0.065, 95% CI: 0.405–0.658, $p = 0.607$) and for HbA1c was 0.743 (SE: 0.054, 95% CI: 0.636–0.849, $P < 0.001$). GDM, gestational diabetes mellitus; HbA1c, glycated haemoglobin; GA, glycated albumin; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; AUC, area under the ROC curve; SE, standard error; CI, confidence interval

and 2hPG; HbA1c correlated significantly with FPG and 2hPG on women without GDM ($r = 0.294$, $p = 0.001$ and $r = 0.279$, $p = 0.002$, respectively). Correlations between GA, HbA1c, FPG, 1hPG and 2hPG by GDM status are presented in Table 2.

The performances of GA, HbA1c and FPG for the diagnosis of GDM by the OGTT are shown in Fig. 1. According to ROC analysis (Fig. 1), the overall accuracy of GA to diagnose GDM is very low showing that GA does not have ability to correctly discriminate those with and without GDM. The AUC for GA was 0.531 (95% CI: 0.405–0.658; $p = 0.607$). The equilibrium cut-off value for GA was 12.7%; sensitivity and specificity in this cut-off point were 53.6% and 53.3%, respectively (Table 3). GA ≥ 12.7% yielded LR + and LR- of 1.15 and 0.87, respectively. However, GA higher than 15% showed very high

Table 3 Performance of different cut-offs of GA, HbA1c and FPG to diagnose GDM ($n = 149$)

	Threshold	Sensitivity (%)	Specificity (%)
GA (%)	12.0	71.4	29.2
	12.7	53.6	53.3
	13.0	42.9	61.7
	13.5	28.6	77.5
	14.0	21.4	88.3
	14.5	10.7	94.2
	15.0	3.6	98.3
	15.2	3.6	99.2
HbA1c [mmol/mol; (%)]	26 (4.5)	100.0	5.0
	31 (5.0)	78.6	48.8
	37 (5.5)	50.0	86.8
	45 (6.3)	3.6	100
FPG (mg/dl)	80.5	85.7	59.5
	85.5	75.0	90.1
	89.5	64.3	97.5
	92.5	57.1	100
	95.5	39.3	100

GDM gestational diabetes mellitus, *HbA1c* glycated haemoglobin, *GA* glycated albumin, *FPG* fasting plasma glucose

specificity (>98%) to identify GDM, with LR + and LR- of 2.14 and 0.98, respectively. The AUC for HbA1c was 0.743 (95% CI: 0.636–0.849; $p < 0.001$). The difference between AUC for GA and HbA1c was 0.212 (95% CI: 0.068–0.355; $p < 0.004$). FPG had the highest AUC [0.865 (95% CI: 0.772–0.958; $p < 0.001$)] for the detection of GDM than the AUCs of GA and HbA1c, though the difference between AUC for FPG and HbA1c was not statistical significant [0.122 (95% CI: 0.010–0.254; $p < 0.070$)].

Discussion

In this study, we evaluated the performance of GA in the diagnosis of GDM by OGTT as the reference test. Our results showed that GA has a poor overall accuracy to diagnose GDM without the ability to correctly discriminate women with and without GDM.

Our study is in agreement with two previous studies that also reported that GA was not suitable as a diagnostic tool for GDM [21, 22]. In the first study, a cross-sectional case-control study [21], examined 80 Turkish pregnant women and reported AUC of 0.550, similar to the AUC in our study. In the second study, that examined 665 Chinese pregnant women [22], the AUC for the detection of GDM was 0.568. Similar to the Chinese study [22], our findings showed that FPG has a higher diagnostic value than GA and HbA1c for the detection of GDM. On the other hand, the results of Saglam et al. study reported no difference in the AUCs of GA and HbA1c [21]. By contrast, the present study found that AUC for GA in the diagnosis of GDM by the OGTT was much lower than for HbA1c ($p = 0.004$).

Studies performed in the general population have usually reported better accuracy of GA in the diagnosis of diabetes with AUCs ranging from 0.70 and 0.90 [15–20]. This performance is similar to that of HbA1c in most studies. Therefore, these studies have advocated that GA may be a useful alternative to HbA1c under conditions wherein the latter does not reflect glycaemic status accurately.

GA is a measure of glycaemia based on the amount of glucose present in the blood attached to albumin, rather than to haemoglobin. Thus, GA is haemoglobin/erythrocyte independent, consequently, its measurement appears to be more appropriate in people with anaemia, a condition usually seen in pregnant women.

In this study, GA was found to be associated only with HbA1c in women with GDM. GA levels were not associated with glycaemic tests (FPG, 1hPG and 2hPG). In contrast, HbA1c was associated with FPG and 2hPG in women without GDM. These results may suggest that, in general, pregnancy may be considered as a confounding factor when assessing glycaemic status using GA. Yi et al. already reported that GA levels decreased as pregnancy progress with or without GDM [27]. Hiramatsu et al. had shown similar results in healthy pregnant women [28]. Li et al. also related that GA levels reduce continually as pregnancy progressed in both women with and without GDM [29]. Nonetheless, they observed that elevated GA levels had a positive association with the incidence of babies with birthweights ≥3.5 g and macrosomia in GDM women with poor glycaemic control [29]. In two similar studies, Mendes et al. showed that GA, besides from providing additional information to HbA1c, when used separately, performed better than traditional biomarkers in predicting neonatal birthweight and large-for-date babies in pregnant women with GDM [30, 31]. Caution should therefore be advised in interpreting GA measurements during pregnancy. The reasons why and how GA decreases from early to late pregnancy are yet to be elucidated.

Although this is out of the scope of this study, we analysed our data to explore the association of GA and body weigth. There was no association between GA and current body weigth or body mass index in our pregnant women (result not shown). We think this topic is clinically relevant and properly designed studies are needed to evaluate the influencing factors of GA in pregnant women.

The present study has some strength. As far as we know is the first to relate the performance of GA to diagnose GDM in Brazilian women. Women with disorders that could interfere on albumin metabolism and influence on GA levels were excluded. We attempted to follow the STARD

2015 reporting guideline for diagnostic accuracy studies to assure reporting the results adequately. The study also has limitations. The sample size is small, but it was calculated a priori to assure the study power of 80% and an estimated alfa error of 5%. Besides, patients were consecutively enroled, and the sample reflects the prevalence of GDM in our population, as recommended in diagnostic accuracy study [32]. We use a single dosage of GA to ascertain whether measurement of GA can be employed to diagnose GDM, however, the chosen period at 24–28 weeks of gestation matches with the period of increased insulin resistance caused by placental hormones. In addition, we were not able to evaluate any relationship between GA levels and neonatal complications due to the cross-sectional study design.

In conclusion, serum GA at 24–28 weeks of gestation does not have enough diagnostic accuracy to correctly discriminate those with and without GDM. In summary, our results do not support the solely use of GA at 24–28 weeks of gestation in the diagnosis of GDM. The potential of GA in pregnancy remains unknown. Further studies are necessary to determine the value of testing GA in pregnant women and the effects of pregnancy on GA merit consideration when using GA as an indicator of glycaemic control in clinic.

Data availability

The datasets generated during the current study are available from the corresponding author on request.

Author contributions Conceived and designed the experiments: FCC, PBR, JLC. Contributed materials and reagents: FCC, PBR, JLC. Performed the experiments: FCC, PBR, MKH, PACF. Acquired the data: PBR, MKH. Analysed and interpreted the data: FCC, PBR, JLC. Drafted the article/wrote the paper: FCC, PBR, JLC. All authors have accepted responsibility for the entire content of this manuscript and approved its submission. FCC and PBR should be considered joint first author.

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Compliance with ethical standards

Conflict of interest The authors declare no competing of interests.

Ethics approval The present study protocol followed the institutional policies that are in compliance with the Declaration of Helsinki. The protocol was approved prior to commencing by the Ethics Committee of the Hospital de Clinicas de Porto Alegre (GPPG-HCPA) (registration number GPPG 2018–0409).

Informed consent and consent for publication Informed consent providing clearance for the use of stored material, data and their publication after anonymization was obtained from all individual participants included in the study.

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