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Métodos para avaliação e acompanhamento de pacientes candidatos a terapia de substituição de células-β.

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À minha família por todo amor e suporte.

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“Para nós os grandes homens não são aqueles que resolveram os problemas,
mas aqueles que os descobriram”.

Albert Schweitzer

O formato da dissertação o segue o modelo recomendado pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, da Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma breve introdução sobre o tema, seguido de dois artigos originais contendo os resultados, finalizando com as considerações finais e perspectivas deste trabalho.

RESUMO

O tratamento intensivo do Diabetes Mellitus Tipo 1 (DM1), objetivando atingir glicemias próximas do normal, reduz as complicações microvasculares e cardiovasculares, porém está associado ao aumento de episódios de hipoglicemias graves em até 3 vezes. A hipoglicemia é frequentemente negligenciada e suas complicações nem sempre são avaliadas. O National Institute for Health and Care Excellence orienta que os pacientes diabéticos sejam avaliados para o risco de hipoglicemia usando questionários específicos. Pacientes com percepção reduzida a hipoglicemia (PRH) tem risco aumentado de hipoglicemias graves e esses episódios podem evoluir para perda de consciência, convulsões, coma e até morte.

A terapia de substituição de células β, através de transplante de ilhotas ou pâncreas, é uma opção de tratamento para pacientes com hipoglicemias chamadas problemáticas. As hipoglicemias problemáticas são definidas por dois ou mais episódios de hipoglicemia grave por ano ou um episódio associado a PRH. O acompanhamento desses pacientes é desafiador, tanto pelas próprias características da doença quanto pela limitação dos métodos utilizados para monitorar o controle glicêmico. Esses pacientes, na sua maioria, apresentam doença renal crônica, com anemia e uremia, levando a interferências na dosagem da hemoglobina glicada (HbA1C). Já o automonitoramento da glicemia capilar apresenta baixa aderência, com número de medidas diárias muito aquém das necessárias para um adequado ajuste e controle glicêmico.

Diante do exposto, nós realizamos inicialmente a validação, adaptação transcultural e tradução de questionários para avaliar hipoglicemia (Clarke, Gold e Escala de Sintomas de Hipoglicemia de Edimburgo). A metodologia seguiu as orientações do ISPOR's Task Force for Translation and Cultural Adaptation. Os parâmetros de consistência interna, confiabilidade (teste-reteste) e validade convergente foram adequadamente alcançados. Os questionários de Clarke e Gold foram posteriormente utilizados para avaliar a prevalência de PRH em um ambulatório de atendimento a pacientes com DM1 do Hospital de Clínicas de Porto Alegre. A prevalência de PRH nos 123 pacientes incluídos foi de 38,3% com o questionário Clarke e de 25,2% com o questionário Gold. Esta prevalência foi maior nos pacientes com maior tempo de diabetes (p=0.002), menor HbA1c (p=0.047) e menor taxa de filtração glomerular (p=0.001).

Um segundo estudo foi realizado em pacientes com DM1 candidatos a terapia de substituição de células β, para avaliar o desempenho do sistema flashGM tendo como método de referência o automonitoramento da glicemia capilar (SMBG). Trinta e nove pacientes foram incluídos e 1420 pares de medições de glicose foram geradas durante o período de 14 dias. 41,5% dos pacientes estavam em terapia dialítica e os demais eram pacientes transplantados

renais. 59% dos pacientes foram diagnosticados com hipoglicemia problemática. Correlação significativa foi observada entre os dois métodos (r = 0,818, p <0,001). A análise de Bland-Altman evidenciou uma diferença média entre os níveis de glicose de 2,32 mg / dL, porém com limites de confiança variando entre –122 a 127 mg/dL. A média absoluta relativa da diferença (MARD) entre o sistema flashGM e o SMBG foi de 25,28% (IC95% 23,6-27,5%), valor mais alto que o considerado adequado para medida de acurácia entre métodos. Quando as medidas de glicose pareadas foram plotadas no Clarke Error Grid, 92,75% delas encontravam-se na zona de precisão aceitável (zonas A e B). Os pacientes verificaram a glicose 2,8 vezes mais com o sistema flashGM do que com o SBGM.

ABSTRACT

Intensive treatment of T1DM, aiming to achieve near-normal glycemia, reduces microvascular and cardiovascular complications, but is associated with an increase in episodes of severe hypoglycemia up to three times. Hypoglycemia is often neglected and its complications are not always evaluated. The National Institute for Health and Care Excellence recommends that diabetic patients should be evaluated for the risk of hypoglycemia using specific questionnaires. Patients with impaired awareness of hypoglycemia (IAH) are at increased risk of severe hypoglycemia and these episodes may progress to loss of consciousness, seizures, coma, and even death.

B-cell replacement therapy, via islet or pancreas transplantation, is a treatment option for patients with so-called problematic hypoglycemia. Problematic hypoglycemia is defined by two or more episodes of severe hypoglycemia per year or an episode associated with IAH. The follow-up of these patients is challenging, both for the characteristics of the disease itself and for the limitation of the methods used to monitor glycemic control. These patients, for the most part, have chronic kidney disease, with anemia and uremia, leading to interferences in the dosage of HbA1C. Self-monitoring of capillary glycemia has low adherence, with a number of daily measurements far below those required for adequate glycemic control and adjustment.

In light of the above, we initially performed validation, cross-cultural adaptation and questionnaire translation to assess hypoglycemia (Clarke, Gold and Edinburgh's Hypoglycemia Symptom Scale). The methodology followed the guidelines of ISPOR's Task Force for Translation and Cultural Adaptation. The parameters of internal consistency, reliability (test-retest) and convergent validity were adequately achieved. The Clarke and Gold questionnaires were later used to evaluate the prevalence of HRP in an outpatient clinic for patients with DM1 at Hospital de Clínicas de Porto Alegre. The prevalence of HRP in the 123 patients included was 38.3% with the Clarke questionnaire and 25.2% with the Gold questionnaire. This prevalence was higher in patients with greater diabetes time, lower HbA1c and lower glomerular filtration rate.

A second study was carried out in patients with DM1 candidates for β-cell replacement therapy, to evaluate the performance of the flashGM system using as reference method the self-monitoring of capillary glycemia (SMBG). Thirty-nine patients were enrolled and 1420 pairs of glucose measurements were generated over the 14 day period. 41.5% of the patients were in dialysis therapy and the remainder were renal transplant patients. Fifty-nine percent of the

patients were diagnosed with problematic hypoglycemia. Significant correlation was observed between the two methods ($r = 0.818$, $p < 0.001$). The Bland-Altman analysis showed a mean difference between glucose levels of 2.32 mg / dL, but with confidence limits ranging from -122 to 127 mg / dL. The mean relative absolute difference (MARD) between the flashGM system and the SMBG was 25.28% (IC95% 23.6-27.5%), a higher value than that considered adequate for measuring accuracy between methods. When paired glucose measurements were plotted on the Clarke Error Grid, 92.75% of them were in the acceptable precision zone (zones A and B). The patients verified the glucose 2.8 times more with the flashGM system than with the SBGM.

LISTA DE ABREVIATURAS E SIGLAS

INTRODUÇÃO

DM	Diabetes mellitus
DM1	Diabetes mellitus tipo 1
DRC	Doença Renal Crônica
ESHE	Escala de Sintomas de Hipoglicemia de Edimburgo
FlashGM	Sistema flash de monitorização de glicose
HbA1c	Hemoglobina glicada
IAH	*"Impaired awareness of hypoglycemia"*
PRH	Percepção reduzida a hipoglicemia

ARTIGOS

CEG	Consensus (Parkes) Error Grid
CGMS	Continuous glucose monitoring system
CKD	Chronic kidney disease
DM	Diabetes mellitus
eGFR	Estimate glomerular filtration rate
EHSS	Edinburgh Hypoglycemia Symptom Scale
FlashGM	Flash glucose monitor
HbA1c	Glycated hemoglobin
HPLC	High-performance liquid chromatography
IAH	Impaired awareness of hypoglycemia
MAD	Mean absolute difference
MARD	Mean absolute relative difference
MD	Mean difference
SD	Standard deviation
SMBG	Self-monitoring of blood glucose
T1DM	Type 1 Diabetes
T2DM	Type 2 Diabetes

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Introdução

O Diabetes mellitus tipo 1 (DM1) é causado pela destruição autoimune das células β pancreáticas, levando a deficiência da produção de insulina, sendo responsável por 5-10% dos casos de diabetes (1). O tratamento intensivo do DM1, objetivando atingir glicemias próximas do normal, reduz as complicações microvasculares e cardiovasculares, porém está associado ao aumento de episódios de hipoglicemias graves em até 3 vezes (2, 3).

Hipoglicemia clinicamente relevante é definida como glicemia abaixo de 54mg/dl e hipoglicemia grave é aquela em que o paciente necessita auxilio de terceiros para realizar seu tratamento (4). Pacientes com hipoglicemia grave tem um risco de morte 3,4 vezes maior que pacientes com hipoglicemias leves (5) e é estimado que hipoglicemia seja a causa de morte em até 10% dos pacientes com DM1 (6).

Mecanismos contrarregularatórios da hipoglicemia

Com a redução sérica da glicemia, mecanismos de contra regulação são ativados para evitar episódios de hipoglicemia. O primeiro deles é a redução da secreção de insulina, que nos pacientes usuários de insulina não ocorre, pois a mesma é administrada regularmente de forma exógena (7). O segundo mecanismo é a secreção de hormônios contrarreguladores como glucagon, adrenalina, cortisol e GH (8).

A adrenalina é responsável por causar os sintomas autonômicos da hipoglicemia como tremores, palpitações e sudorese. Com a evolução do DM, a secreção de hormônios contrarreguladores vai reduzindo, estando principalmente relacionado com o tempo de uso de insulina. Esta redução é responsável pela diminuição ou ausência dos sintomas adrenérgicos num episódio de hipoglicemia (9, 10).

Em pacientes que apresentam hipoglicemia recorrente, a redução da secreção desses hormônios é ainda mais acentuada, pois eles apresentam uma resposta cerebral reduzida a neuroglicopenia (11).

Os pacientes mais propensos a terem hipoglicemias são aqueles com história de evento de hipoglicemia no último mês, extremos de idade, com nível educacional mais baixo, e portadores de comorbidades como doença renal crônica, doenças vasculares, síndromes de má absorção intestinais e uso de medicações como betabloqueadores (7, 11-13). No entanto o maior fator de risco para hipoglicemia grave é a percepção reduzida a hipoglicemia (7), particularmente comum em pacientes diabéticos de longa data e com menos sintomas autonômicos de hipoglicemia (14, 15).

Hipoglicemias Problemáticas

Alguns pacientes tem hipoglicemias chamadas problemáticas, definidas por dois ou mais episódios de hipoglicemia grave por ano ou um episódio associado a percepção reduzida a hipoglicemia, elevada variabilidade glicêmica ou medo e comportamento inadequado a hipoglicemia (16).

Percepção reduzida a hipoglicemia (PRH), do inglês "*impaired awareness of hypoglycemia" (IHA)* é denominada a capacidade reduzida ou ausente do paciente de reconhecer os sintomas de hipoglicemia. Esta condição está associada a um aumento de 6 vezes no risco de hipoglicemias graves (17) e estima-se que 20-25% dos pacientes com DM1 apresentem PRH (18, 19).

Consequências da Hipoglicemia

As hipoglicemias, quando leves, podem causar sintomas desconfortáveis e efeitos negativos no humor, entretanto quando o paciente apresenta neuroglicopenia, tem sua capacidade de concentração prejudicada, levando a redução da produtividade no trabalho, redução da habilidade de dirigir e aumento do risco de quedas por alteração da coordenação e equilíbrio (20, 21). Em casos de neuroglicopenia severa o paciente pode apresentar convulsões e até coma (22).

Hipoglicemias também podem interferir na repolarização ventricular, alterando o segmento ST e o intervalo QT, com consequente aumento no risco de arritmias. (23). Além disso, as catecolaminas e outros hormônios secretados na hipoglicemia causam aumento da viscosidade sanguínea e coagulação, influenciando a circulação sanguínea (24). Essas alterações podem explicar a etiologia das mortes súbitas durante o sono ("Dead in Bed Syndrome") nos pacientes com DM e o efeito paradoxal encontrado no estudo ACCORD (25), onde controle glicêmico intensivo levou a aumento da mortalidade.

A hipoglicemia também altera o fluxo sanguíneo cerebral, e em pacientes com hipoglicemias recorrentes, esse fluxo pode permanecer alterado mesmo em situações de euglicemia(26). Acidente vascular isquêmico transitório também tem sido reportado como manifestação de hipoglicemia (27).

Os extremos de idades são mais suscetíveis aos efeitos deletérios da hipoglicemia sobre a cognição (21). Pacientes que tiveram hipoglicemias graves repetidas antes dos 5 anos de idade, quando adultos, tiveram escores mais baixos em testes de cognição do que adultos que,

enquanto crianças diabéticas, não tiveram esses episódios (28). Em idosos, a exposição a episódios de hipoglicemia severa também está associada a declínio cognitivo e demência (29).

Os episódios recorrentes de hipoglicemias também levam a consequências além de seus efeitos imediatos. Um dos mais impactantes e deletérios é o medo de hipoglicemias que afeta tanto pacientes quanto seus familiares e influencia negativamente na aderência ao tratamento e no adequado controle glicêmico (30).

Pelo exposto, vemos que reconhecer os sintomas de hipoglicemia é de extrema importância para possibilitar o tratamento e evitar episódios graves. O diagnóstico de PRH permite que o tratamento e alvos glicêmicos sejam ajustados para reduzir o risco de hipoglicemia (31).

Avaliação das Hipoglicemias

O National Institute for Health and Care Excellence (32) orienta que os pacientes diabéticos sejam avaliados para o risco de hipoglicemia usando questionários específicos como os de Clark (33) e Gold (17). Já para avaliação e classificação dos sintomas hipoglicêmicos, a Escala de Sintomas de Hipoglicemia de Edimburgo é empregada (34).

O questionário de Clarke (33) é composto por 8 questões de múltipla escolha referentes a percepção e frequência dos sintomas associados aos episódios de hipoglicemias, bem como valores de glicemia que desencadeiam sintomas. Cada questão é classificada como "percepção normal" ou "percepção reduzida". Quatro ou mais respostas "percepção reduzida" caracterizam PRH.

O questionário de Gold (17) utiliza uma escala de Likert com 7 pontos (1= sempre sente a hipoglicemia, 7 = nunca sente a hipoglicemia) para responder a questão: "Você sabe quando as suas hipoglicemias estão começando?". Um escore de 4 a 7 é compatível com PRH.

A Escala de Sintomas de Hipoglicemia de Edimburgo (34) é um instrumento para avaliar os sintomas dos pacientes em um episódio típico de hipoglicemia. É composta de 11 sintomas divididos em 3 domínios: autonômico, neuroglicopênico e mal-estar, que é avaliado por uma escala de Likert de 7 pontos, sendo 1= não tem o sintoma e 7= o sintoma é muito forte.

Esses questionários, além de úteis na prática clínica por auxiliar na identificação dos pacientes com PRH e possibilitar o ajuste do tratamento para reduzir o risco de hipoglicemias graves, também são úteis no campo da pesquisa, permitindo que se compare objetivamente diferentes populações. Schopman *et al.*, avaliando pacientes com DM1, evidenciou que

pacientes com PRH, avaliados pelo questionário de Gold, apresentaram 2 vezes mais hipoglicemias bem como mais hipoglicemias assintomáticas, em um acompanhamento de 4 semanas, do que os pacientes com percepção normal (35). Geddes *et al*. reportou resultados semelhantes de prevalência de PRH, quando utilizados os questionários de Clarke e Gold em pacientes com DM1(36). Em outro estudo de Guedes *et al*., pacientes com PRH avaliados pelo questionário de Gold apresentaram menor intensidade de sintomas autonômicos, avaliado pela Escala de Sintomas de Hipoglicemia de Edimburgo, nos episódios de hipoglicemias (37).

Monitorização do controle glicêmico

As diretrizes atuais recomendam a dosagem da hemoglobina glicada (HbA1C) e o automonitoramento da glicemia capilar para acompanhamento do controle glicêmico e avaliação do risco de complicações crônicas relacionadas ao diabetes (38, 39).

A HbA1C é formada por reações não enzimáticas de glicação na hemoglobina, refletindo a glicemia dos últimos 120 dias. Ela possui forte associação com os desfechos clínicos no DM e apresenta uma excelente padronização de seus métodos analíticos. Entretanto, diversas situações clínicas que modificam o tempo de vida dos eritrócitos, alteram a glicosilação da hemoglobina que interfere diretamente nos níveis da HbA1C, dificultando a interpretação do exame (40-42). Anemia, uremia, coleta de sangue frequentes e o uso de eritropoietina, presentes na maioria dos pacientes candidatos a transplante, são exemplos dessas situações clínicas (43).

O automonitoramento da glicemia capilar, permite que o paciente verifique sua glicose e faça ajustes em seu tratamento insulínico e dieta, melhorando seu controle glicêmico. Diferentes diretrizes mundiais recomendam que o automonitoramento da glicemia capilar seja realizado 4 a 10 vezes ao dia, incluindo medições antes das refeições, lanches e antes de dormir (38, 44). Estudos tem demostrado que quanto maior o número de medidas realizada pelo paciente, melhor o seu controle glicêmico (45, 46).

Visto que a HbA1C pode sofrer várias interferências na população de pacientes com doença renal crônica (DRC) e que grande parte dos pacientes diabéticos não realizam com frequência adequada os testes de glicemia capilar, torna-se necessário o estudo de outros métodos mais efetivos para monitorar a glicemia.

Recentemente, um novo dispositivo de medida da glicemia intersticial tem sido utilizado para monitoramento do controle glicêmico. Trata-se do sistema flash de monitorização de

glicose (flashGM). Ele é composto de um sensor que mede a glicose intersticial a cada 15 minutos e guarda as informações por até 8 horas. Ele é aplicado na parte superior/posterior do braço com o uso de um aplicador e pode ser mantido por até 14 dias. Para visualizar o valor da glicose utiliza-se um leitor para digitalizar as informações do sensor. O leitor tem a capacidade de guardar informações de até 90 dias e fornecer gráficos das medidas da glicose. Esse sistema difere dos anteriores por não requerer calibração com testes de ponta de dedo (Figura 1).

Alguns estudos têm demostrado desempenho semelhante do sistema flashGM, quando comparado com glicemias capilares de ponta de dedo (8 testes/dia) e outros sensores contínuos de glicose (47, 48). O uso do flashGM também tem sido associado com redução na incidência e no tempo de exposição à hipoglicemias, além de melhorar a adesão nas medidas de automonitoramento da glicose (49, 50). No entanto, este método ainda não foi testado em pacientes com alta variabilidade glicêmica e doença renal crônica.

Figura 1: Sistema de monitoramento de glicose intersticial. **(A)** Sensor e aparelho de leitura da glicose; **(B)** aplicação do sensor com dispositivo específico do sistema; **(C)** leitura da glicose – passagem do leitor sobre o sensor; **(D)** glicemia medida do paciente.

Manejo das Hipoglicemias Problemáticas

Para pacientes com DM1 de difícil controle glicêmico e hipoglicemias problemáticas, a despeito do melhor tratamento clínico e multidisciplinar realizado, a terapia de substituição de células β, seja através do transplante de pâncreas ou de ilhotas pancreáticas, é uma importante opção terapêutica, já que é capaz de restaurar a produção endógena de insulina e a percepção dos sintomas de hipoglicemia. (51).

O objetivo da terapia de substituição de células β é alcançar a independência de insulina. No transplante de pâncreas bem-sucedido, este objetivo é frequentemente alcançado (52). Já no transplante de ilhotas pancreáticas, nem sempre este objetivo é plenamente alcançado, especialmente na primeira infusão de ilhotas. Mesmo assim, pela presença de peptídeo C e consequente produção de insulina, mesmo que parcial, esta terapia é capaz de reduzir a frequência e a gravidade dos episódios de hipoglicemias, e permite a redução das doses de insulina levando a uma melhora significativa do controle glicêmico dos pacientes (53-55). Alguns estudos relatam desaceleração das complicações microvasculares e até mesmo melhora da nefropatia diabética após essa terapia (56-58).

Justificativa

Considerando a alta frequência de percepção reduzida a hipoglicemias em pacientes com DM1, bem como os riscos associados a estes episódios, a tradução, adaptação transcultural e validação para a língua portuguesa de questionários para avaliação da percepção de hipoglicemias é de extrema valia. Estes instrumentos podem auxiliar na identificação de pacientes sob risco elevado de hipoglicemias problemáticas e auxiliar no manejo terapêutico desta população. Também serão de grande utilidade em pesquisas clínicas brasileiras.

Neste contexto, um grupo de pacientes muito suscetível a apresentar percepção reduzida a hipoglicemias são os pacientes com DM1 e doença renal crônica candidatos a transplante de células β. Nesta população, o uso de sistemas de automonitoramento da glicemia que aumentam a adesão às verificações de glicemias, auxiliando na identificação precoce de tendências à hipoglicemias e hipoglicemias com percepção reduzida ou ainda excursões rápidas da glicemia são muito necessárias. Porém, até o presente momento, estudos de avaliação da performance do flashGM em relação a um método de referência não foram descritos para esta população de pacientes.

Objetivos

Os objetivos desta dissertação são:

1. Realizar a tradução, adaptação transcultural e validação para a Língua Portuguesa de três questionários de avaliação de presença e sintomas de hipoglicemia: questionários de Gold e Clarke e a Escala de Hipoglicemia de Edimburgo;

2. Avaliar, através dos questionários validados, a prevalência de percepção reduzida a hipoglicemia em um ambulatório de atendimento a pacientes com DM1 no Hospital de Clínicas de Porto Alegre;

3. Avaliar o desempenho de um sistema de automonitoramento glicêmico através da medida da glicemia intersticial (sistema flashGM), tendo como referência o teste de automonitoramento da glicemia capilar, em pacientes candidatos a terapia de substituição de célula β.

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Capítulo 1 – Artigo Original 1

Hypoglycemia symptoms and awareness of hypoglycemia in type 1 Diabetes Mellitus: cross-cultural adaptation and validation of Portuguese version of three questionnaires and evaluation of its risk factors.

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Hypoglycemia symptoms and awareness of hypoglycemia in type 1 Diabetes Mellitus: cross-cultural adaptation and validation of Portuguese version of three questionnaires and evaluation of its risk factors.

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ABSTRACT

Aims: To adapt and validate the Clarke and Gold questionnaires and the Edinburgh Hypoglycemia Symptom Scale (EHSS) to Brazilian Portuguese and to determine the prevalence and risk factors associated with impaired awareness of hypoglycemia (IAH) in patients with type 1 diabetes mellitus (T1DM).

Methods: The process of translation, cultural adaptation, and validation of the questionnaires followed the recommendations of the ISPOR -Task Force for Translation and Cultural Adaptation. Patients with T1DM for a minimum of 12 months, aged 18 years or older, and with Brazilian nationality were selected to participate.

Results: 123 patients were enrolled. The Clarke and Gold questionnaires as well as the EHSS exhibited adequate internal consistency, test-retest reliability, and convergent validity. The prevalence of IAH was 38.3% with the Clarke questionnaire and 25.2% with the Gold questionnaire. The prevalence increased with longer duration of diabetes, lower HbA1c, and lower eGFR.

Conclusions: The validation and cross-cultural adaptation of the proposed questionnaires to Brazilian Portuguese were adequate. In this sample of T1DM, the prevalence of IAH was high and associated with a longer duration of T1DM, lower HbA1C and lower eGFR.

Keywords: hypoglycemia; diabetes mellitus; Clarke questionnaire; Gold questionnaire; Edinburgh Hypoglycemia Symptom Scale; cross-cultural adaptation; validation.

1. Introduction

Hypoglycemia is a common side effect of insulin secretagogue agents or insulin itself and presents a major barrier to optimal glycemic control since treatment intensification often increases hypoglycemic events (1). According to the International Hypoglycaemia Study Group, relevant hypoglycemia is defined as a glucose concentration below 3.0 mmol/L (54 mg/dL) (2). Severe hypoglycemia is defined as cognitive impairment requiring assistance from another person and may progress to loss of consciousness, seizures, coma, or death (3). McCoy et al. showed that patients with severe hypoglycemia had a 3.4-fold higher risk of death compared to those with no or mild hypoglycemia (4). It is estimated that hypoglycemia is the direct cause of death in 4-10% of patients with type 1 diabetes mellitus (T1DM) (5).

Recognizing warning symptoms of hypoglycemia is critical in order for patient's self-treatment to avoid severe hypoglycemic episodes. Impaired awareness of hypoglycemia (IAH) is a syndrome in which the ability to detect warning symptoms are reduced or absent. These patients exhibit a nearly six-fold higher frequency of severe hypoglycemia than patients without IAH (6).

Approximately 20-25% of adults with T1DM have IAH (7, 8). Schopman et al. showed that patients with IAH had a two-fold greater total frequency of hypoglycemic episodes over a four-week monitoring period and significantly more episodes of asymptomatic hypoglycemia when compared with patients in a normal awareness of hypoglycemia (i.e., non-IAH) group (47% and 14%, respectively) (9).

The National Institute for Health and Care Excellence suggests that diabetic patients should be assessed for hypoglycemia risk using specific questionnaires to identify those at highest risk for severe hypoglycemia (10). This diagnosis allows re-evaluation of the glycemic target and modifications in treatment to reduce hypoglycemia risk and its complications(31). Scoring systems such as the Clarke (12) and Gold (6) questionnaires represent some of the available instruments used to identify patients with IAH. The Edinburgh Hypoglycemia Symptom Scale (EHSS) is another instrument to evaluate symptoms in a typical hypoglycemic episode and helps to characterize patients with IAH(13,14).

Based on the aforementioned importance of validated instruments to identify IAH and the fact that no such instruments adapted to Brazil are currently available, the aim of this study was to adapt and validate the Clarke and Gold questionnaires and the EHSS to Brazilian Portuguese. Another objective of this study was to determine the prevalence of IAH in patients

with T1DM attending a tertiary hospital outpatient clinic by comparing the performance of the three-hypoglycemia questionnaires in this population. Finally, we aimed to characterize the clinical and laboratory profiles of such patients as well as the variables associated with IAH.

2. Subjects, Material and Methods

2.1. Participants

This study was carried out in a diabetes outpatient clinic of a tertiary hospital in Brazil. Eligibility criteria included aged ≥18 years; previous diagnosis of T1DM; disease duration of 12 months at minimum; and Brazilian nationality. The exclusion criteria included developmental disabilities or psychiatric disorders that would pose an obstacle in completing the structured interview. Demographic (age, gender), anthropometric (body mass index [BMI]), clinical characteristics (disease duration, type of treatment, presence of diabetic chronic complications, hypoglycemia frequency) and laboratory (fasting plasma glucose, HbA1c, and creatinine to estimate glomerular filtration rate [eGFR]) data were collected from patients' electronic records. The Institutional Ethics Committee (GPPG # 13-0210) approved this study.

2.2. Questionnaires

The Clarke questionnaire (12) is a frequently used instrument to evaluate IAH. It comprises eight questions regarding the patient's perception of hypoglycemia, the frequency of hypoglycemic episodes, and a subjective estimation of the glycemic threshold for symptom generation. Each answer is classified as either normal awareness (A) or reduced awareness (R). Four or more answers marked as R categorizes a subject as having IAH.

The Gold questionnaire (6) uses a simple 7-point Likert scale (1 = Always aware of hypoglycemia, 7 = Never aware of hypoglycemia) to answer the question "Do you know when your hypoglycemia is starting?". A score between 4 and 7 is compatible with IAH.

The EHSS(14) is an instrument to evaluate patients' experiences of symptoms in a typical hypoglycemic episode. It comprises 11 symptoms divided into three domains—neuroglycopenic, autonomic, and malaise, which are evaluated by a 7-point Likert scale "1 = Not at all, 7 = Very severely".

2.3. Translation and cultural adaptation

The translation and cultural adaptation process followed the recommendations of ISPOR's Task Force for Translation and Cultural Adaptation (15). The researchers obtained permission from the main authors of the Clarke and Gold questionnaires and the EHSS to translate, adapt in a cross-cultural manner, and validate the instruments for use in Brazilian Portuguese.

Two independent translators who are native speakers of Brazilian Portuguese and fluent in English performed the initial forward translation of the original instruments into Brazilian Portuguese. This step resulted in two Brazilian Portuguese versions that were subsequently analyzed by an expert committee composed of endocrinologists, linguists, and the two initial translators. The committee identified and corrected by consensus any ambiguities and discrepancies of words, sentences, and meanings. This process, called reconciliation, generated the preliminary translated version of each instrument.

The Brazilian Portuguese version was back translated into English by two independent translators who were native English speakers and fluent in Brazilian Portuguese. The two back-translated versions were then analyzed by the expert committee to identify and correct by consensus any discrepancies in format, wording, syntax, meaning, and relevance.

Based on the translations and back-translations obtained in the previous steps, the expert committee analyzed each item (questions and answers) of the questionnaires to define the most appropriate version in terms of conceptual, semantic, and content equivalency (harmonization step), which produced a final pilot version of the Brazilian Portuguese questionnaires.

The next step comprised of administering a cognitive debriefing test to five outpatients with T1DM, which were of both sexes, varying ages, and different socioeconomic groups in order to ensure the patients were a representative sample of the target population. The principal investigator reviewed the results of the cognitive debriefing to identify translation modifications to facilitate the comprehension of the questionnaires. Proofreading was performed to ensure correction of minor errors prior to the generation of the final version. The final version of the questionnaires was given to a total of 40 patients.

2.4. Data analysis and psychometric measurements

Demographic and clinical data were described using mean (SD) and frequency (percentage). A t-test was used for continuous variables whereas a chi-square test was used for categorical variables. The relation between two numerical variables was measured by

Spearman's correlation coefficient. To evaluate the reliability and validity of the translated instruments, they were applied in 123 patients (including the initial 40 evaluated). Internal consistency was evaluated by Cronbach's α (values above 0.7 were considered acceptable). Exploratory factor analysis was performed on the 11 items from the EHSS and on the seven items from the Clarke questionnaire.

Test-retest reliability was calculated to verify intra-observer variability by estimation of the interclass correlation coefficient (ICC) and t-test between times 1 and 2 for the Gold questionnaire and the EHSS (continuous variables) and with Kappa for the Clarke questionnaire (ordinal variables). Since there is no reference test for evaluating IAH, we used the necessity of IV glucose to treat hypoglycemia to assess convergent validity with Spearman's correlation coefficient. Statistical analyses were performed with SPSS 23.0 and the level of significance was defined as $\alpha = 0.05$.

3. Results

3.1. Translation and cultural adaptation

During the debriefing step, one patient did not understand the meaning of the word "palpitations" in the EHSS therefore the expert committee decided to include an explanation of this word in parentheses following the "palpitation" symptom in the questionnaire. Fourteen of the 40 T1DM patients that completed the pre-test questionnaires had an initial difficulty understanding the Likert scale of the EHSS and the Gold questionnaire. Following further explanation, all were able to answer the questions. The questionnaires averaged <10 minutes to complete.

3.2. Descriptive analysis

The clinical and demographic characteristics of the studied patients were as follows: 39.6 ± 11.5 years old; 51.2% male; duration of diabetes: 22.37 ± 3.47 years; BMI 24.33 ± 3.47 kg/m^2; HbA1C 8.42 ± 1.48%; and insulin dose: 0.71 ± 0.22 UI/kg/day. Hypertension was observed in 41.5% of patients, peripheral symmetric polyneuropathy in 27.6%, and retinopathy in 59.3%. 17.1% of patients had an eGFR between 15-60 mL/min/1.73m^2 and 16.3% had a eGFR lower than 15 mL/min/1.73m^2.

3.3. Psychometric measurements

The final versions of the questionnaires were completed by 123 patients (including the initial 40 evaluated) to evaluate the reliability and validity of the instruments. The overall internal consistency of the Clarke questionnaire was demonstrated by Cronbach's α = 0.804. A separate analysis using one question at a time to evaluate the influence of each item on the internal consistency of the instrument resulted in a Cronbach's α = <0.804 for all analyses. Inter-rater reliability was assessed by Kappa = 0.712 (95% CI, 0.598-0.826). Internal consistency of the Gold questionnaire was not performed because this questionnaire comprises only one question. The analysis of the ICC supported the test-retest reliability of the instrument with high agreement (ICC = 0.824; 95% CI, 0.651-0.911) and the paired t-test did not show a significant difference in the re-test (mean score = 2.83 vs. 2.91, $p = 0.734$).

Regarding the EHSS, the overall Cronbach's α was 0.749. When taken one question at a time to evaluate the influence of each item on the internal consistency of the instrument, only the removal of the item "hunger" slightly increased the Cronbach's α to 0.761. After factorial analyses of the scale we identified four subscales based on the eigenvalues after varimax rotation—neuroglycopenic (confusion, drowsiness, odd behavior, speech difficulty, and incoordination), autonomic (sweating, palpitation, and shaking), malaise (headache and nausea), and hunger **(Table 1)**. Test-retest reliability was performed by subscales, with no difference between means on paired t-test and with ICC (95% CI) as follows: neuroglycopenic = 0.93 (0.72-0.95); autonomic = 0.91 (0.60-0.97); malaise = 0.88 (0.52-0.97) and hunger = 0.75 (0.21-0.96).

Convergent validity was verified by correlation between the presence of IAH (as diagnosed by the Clarke and Gold questionnaires) and the need for IV glucose for hypoglycemia treatment. Clarke's correlation was stronger (r = 0.543; $p = 0.0001$) than Gold's correlation (r = 0.248; $p = 0.006$). In the EHSS, we tested the correlation of the four subscales with the need for IV glucose and with the number of hypoglycemic events in the preceding year. The neuroglycopenic subscale showed a correlation of 0.408 ($p < 0.001$) and 0.410 ($p < 0.001$), respectively **(Figure 1)**. The Clarke and Gold questionnaires showed a moderate correlation between each other (r = 0.661; $p < 0001$).

The Brazilian Portuguese version of the questionnaires are available at **Table 2**.

3.4. Clinical and laboratory characteristics of patients according to hypoglycemic symptoms

From the 123 T1DM patients evaluated, 55.3% (n = 68) had experienced at least one episode of severe hypoglycemia in the previous 12 months. Of them, 31.7% (n = 39) had received IV glucose to treat hypoglycemia. The prevalence of IAH was 38.3% with the Clarke questionnaire and 25.2% with the Gold questionnaire. The prevalence increased with longer duration of diabetes, from 12.5% in patients with <10 years of disease to 44% for those with duration >20 years. It is important to note that 63.4% of the patients have had a diagnosis of diabetes for >20 years. **Table 3** shows the clinical and demographic characteristics of the patients analyzed by the two questionnaires. Patients classified with IAH by the Clarke questionnaire had a significantly longer duration of diabetes (p=0.002), lower HbA1c (p=0.047), and lower eGFR(p=0.001). No such difference was observed using the Gold questionnaire, as shown in **Figure 2**. After multivariate analysis, longer duration of T1DM (*p* = 0.047) and lower eGFR (*p* = 0.014) were shown by the Clarke questionnaire to be independent risk factors for IAH.

4. Discussion

This paper describes the translation, cross-cultural adaptation, and validation of the Brazilian Portuguese version of two instruments (i.e., the Clarke and Gold questionnaires) to assess IAH in patients diagnosed with T1DM and one instrument (i.e., the EHSS) to characterize hypoglycemic events. The psychometric properties, including internal consistency, reliability, and validity, were assessed. These analyses ensured that the process of adaptation and validation was performed in an appropriate manner and they allowed comprehensible and feasible Brazilian Portuguese questionnaires to be devised in order to identify IAH in T1DM patients in clinical practice. Furthermore, we demonstrate a high prevalence of IAH in our sample (25-38%) and found that a longer diabetes duration and lower eGFR are independent risk factors for this outcome.

In our population, the ability of each questionnaire in detecting IAH differed. The Clarke questionnaire diagnosed IAH in more patients than did the Gold questionnaire, which contrasts with the results found by Geddes et al. of similar prevalence (16). A possible explanation for this discrepancy relies on the fact that the Clarke questionnaire is a more complete instrument since it evaluates the patient's exposure to episodes of moderate and severe hypoglycemia and also estimates glycemic thresholds for hypoglycemic responses. The Gold questionnaire,

however, is a single-item questionnaire with a 7-point Likert scale, where 1 represents "Always aware of hypoglycemia" and 7 represents "Never aware of hypoglycemia". During the process of transcultural adaptation of this questionnaire, some patients had difficulties understanding the Likert scale, needing further explanation to be able to answer the question. This fact may, in part, explain the difference in prevalence of IAH between the two questionnaires.

The EHSS is also a 7-point Likert scale (1 = Not at all and 7 = Very severely) where the patients rated the intensity of their symptoms in a typical hypoglycemic episode. Differing from the original study[14] which divided the hypoglycemic symptoms in three subscales (autonomic, neuroglycopenic, and malaise), our data fit more appropriately into four subscales. This decision was based on the factorial analysis of the items, where the "hunger" symptom had higher score when analyzed in isolation than when analyzed in conjunction with the "autonomic" subscale, as suggested by the original study.

In our study, the IAH prevalence was higher than in other studies (17,18). The reasons for this finding might be related to the longer duration of diabetes in our population and the fact that they are treated in a tertiary referral hospital. Higher duration of T1DM and fewer autonomic symptoms were associated with the presence of IAH, as seen in previous studies (17,18). We also found an association between lower eGFR and the presence of IAH in the Clarke but not the Gold questionnaire.

Chronic kidney disease (CKD) is an independent risk factor for hypoglycemia. The reasons for this include decreased renal clearance of insulin, decreased degradation of insulin in peripheral tissues, reduced renal gluconeogenesis, and prolonged half-life of other medications (19). In patients on hemodialysis, hypoglycemia is often seen within 24 h after dialysis and occurs as a result of glucose loss during dialysis (20). However, this effect may also be interpreted as meaning that a high frequency of hypoglycemia is the cause of diabetic kidney disease. This theory, while controversial, states that recurrent hypoglycemia and the consequent release of catecholamines causes arterial stiffness, coagulation abnormalities, and increased inflammatory responses that may lead to renal ischemia and CKD (21).

Patients with predominantly neuroglycopenic symptoms are more likely to have IAH and severe hypoglycemia, as observed in previous studies (22). We noticed that some patients did not consider low glucose values as hypoglycemia if they did not have autonomic symptoms. This misunderstanding may contribute to the development of a severe hypoglycemic episode. The patient's knowledge of the various symptoms of hypoglycemia is crucial to enable its recognition and to reduce episodes of severe hypoglycemia and its consequences (11).

5. Conclusion

The process of validation and cross-cultural adaptation of the proposed questionnaires to Brazilian Portuguese was adequate. In this sample of T1DM, the prevalence of IAH was high and associated with longer duration of diabetes and lower eGFR. The high prevalence of IAH among T1DM subjects draws attention to the need for more vigilant care and increased education of these patients in order to reduce morbidity and mortality rates. These questionnaires are of great value to help health care professionals for facilitating the identification of patients at high risk for IAH and to plan interventional strategies to reduce IAH.

Author Contributions and Acknowledgements

PS, ACB, and CBL designed the study, interpreted the data, and reviewed the manuscript. PS, LSG, and ALMS acquired the data. PS and ACB analyzed the data and drafted the manuscript. All authors approved the final version. We would like to thank Dr. Sandra Pinho Silveiro and Dr. Rogerio Friedman for their collaboration in the first stage of translating the questionnaires.

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Declaration of interest

The authors declare no conflict of interest.

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Table 1. Edinburgh Hypoglycemia Symptom Scale factorial analysis compared with the original study.

	Component							
	Neuroglycopenic		Autonomic		Malaise		Hunger	
	A	B	A	B	A	B	A	B
Incoordination	**.804**	**.569**	.201	.184	-.075	.052		-
Odd Behavior	**.800**	**.719**	-.052	.019	.180	-.088		-
Confusion	**.761**	**.808**	.133	.096	-.026	-.079		-
Speech Difficulty	**.737**	**.699**	.263	.024	.044	.194		-
Drowsiness	**.492**	**.535**	.093	-.092	.198	.253		-
Shaking	.207	.008	**.804**	**.574**	.000	.163		-
Palpitation	-.004	.138	**.719**	**.443**	.262	.418		-
Sweating	.267	.156	**.677**	**.696**	.079	.010		-
Headache	.011	.348	.037	.138	**.823**	**.820**		-
Nausea	.109	-.025	.194	.061	**.766**	**.547**		-
Hunger	.007	.076	.034	.724	.033	000	**.945**	-

Eigenvalues A: present study, B: Original Study. KMO 0.724 ($p<0.001$)

Table 2 - Clinical and demographic characteristics of the patients stratified according awareness of hypoglycemia evaluated by the Clarke and Gold questionnaires.

	Clarke			**Gold**		
	Aware (n=76)	IAH (n= 47)	p	Aware (n=92)	IAH (n= 31)	p
IAH (%)	61.7	38.3		74.8	25.2	
Sex (% male)	33.1	17.9		42.3	8.9	
Age	38.01 (11.16)	42.09 (11.73)	0.060	39.2 (11,25)	40.68 (12.36)	0.558
BMI	24.19 (3.42)	24.56 (3.59)	0.568	24.17 (3.42)	24.81 (3,60)	0.390
Diabetes duration	20.17 (10.21)	25.91 (9.29)	**0.002**	21.53 (10.32)	24.84 (9.64)	0.110
Insulin dose	0.72 (0.23)	0.69 (0.20)	0.467	0.71 (0.22)	0.69 (0.20)	0.640
Glycaemia	191.79 (106.53)	166.43 (102.62)	0.196	192.61 (110.8)	150.9 (80.9)	**0.028**
HbA1C (%)	8.62 (1.52)	8.09 (1.36)	**0.047**	8.52 (1.49)	8.13 (1.41)	0.199
GFRe (ml/min/1,73m^2)	84.05 (36,4)	59.96 (40,68)	**0.001**	75.82 (39.55)	71.97 (40.65)	0.648
Nº of hypoglycemia/week	2.2 (1.53)	3.43 (1.97)	**0.000**	2.38 (1.64)	3.52 (2.03)	**0.007**
Nº severe hypoglycemia in 6m	0.93 (1.82)	3.74 (3.27)	**0.000**	1.43 (2.13)	3.71 (3.81)	**0.003**
Nº severe hypoglycemia in 12m	2.05 (3.97)	7.28 (6.10)	**0.000**	3.12 (4.71)	6.81 (6.70)	**0.001**
Intravenous glucose need	0.11 (0.32)	0.63 (0.48)	**0.000**	0.25 (0.43)	0.5161 (0.50)	**0.006**

Variables are described as mean (SD) or percentage (%).

CLARKE Questionnaire

Original	Translation 1	Translation 2	Reconciliation	Back Translation	Final
1) Check the category that best describes you: (check one only)	Marque a categoria que melhor descreve você (marque apenas uma):	Marque a categoria que melhor descreve você (assinale apenas uma opção):	Marque a alternativa que melhor descreve você (marque apenas uma):	Select the option which best describes you (one only):	Marque a alternativa que melhor descreve você (marque apenas uma):
2) Have you lost some of the symptoms that used to occur when your blood sugar was low?	Você perdeu alguns dos sintomas que costumavam ocorrer quando seu açúcar no sangue esta baixo?	Você perdeu alguns dos sintomas que costumavam ocorrer quando seu açúcar no sangue esta baixo?	Você deixou de ter alguns dos sintomas que costumava ter quando seu açúcar no sangue estava baixo?	Have you stopped experiencing some of the symptoms you used to when your blood sugar level was low?	Você deixou de ter alguns dos sintomas que costumava sentir quando seu açúcar no sangue estava baixo?
3) In the past six months how often have you had moderate hypoglycemia episodes?(Episodes where you might feel confused, disoriented, or lethargic and were unable to treat yourself)	Nos últimos seis meses, com que frequência você teve episódios de hipoglicemia onde você se sentiu confuso, desorientado ou letárgico e foi incapaz de tratar-se sozinho?	Nos últimos seis meses, com que frequência você teve episódios de hipoglicemia nos quais você se sentiu confuso, desorientado ou letárgico (prostrado) e não conseguiu se tratar sozinho?	Nos últimos seis meses, com que frequência você teve episódios de hipoglicemia em que tenha se sentido confuso, desorientado ou apático e não conseguiu se tratar sozinho	In the last six months, how often have you experienced episodes of hypoglycemia that made you feel confused, disoriented or lethargic and could not self-treat?	Nos últimos seis meses, com que frequência você teve episódios de hipoglicemia (açúcar baixo no sangue) em que tenha se sentido confuso, desorientado ou apático e não conseguiu se tratar sozinho
4) In the past year how often have you had severe hypoglycemic episodes? (Episodes whereyou were unconscious or had a seizure and needed glucagon or intravenous glucose)	No último ano, com que frequência você teve episódios de hipoglicemia onde você esteve inconsciente ou teve convulsões e necessitou glucagon ou glicose intravenosos?	No último ano, com que frequência você teve episódios de hipoglicemia onde você ficou inconsciente ou teve convulsões e precisou de glucagon ou de glicose intravenosa?	No último ano, com que frequência você teve episódios de hipoglicemia em que tenha perdido a consciência (desmaiado) ou tido convulsões, precisando de glicose intravenosa (injeção de glicose) ou glucagon?	In the last year, how often have you experienced episodes of hypoglycemia in which you lost consciousness (fainting) or seizures, requiring intravenous treatment (glucose injection) or glucagon?	No último ano, com que frequência você teve episódios de hipoglicemia (açúcar baixo no sangue) em que tenha perdido a consciência (desmaiado) ou tido convulsões, precisando de glicose intravenosa (injeção de glicose na veia) ou glucagon?
5) How often in the last month have you had readings <70 mg/dl with symptoms?	Com que frequência, no último mês, você teve leituras menores do que 70mg/dl com sintomas?	Com que frequência, no último mês, você teve leituras menores do que 70mg/dl com sintomas?	No último mês, com que frequência você teve medidas de glicose menores do que 70mg/dl com sintomas?	In the last month, how often have your glucose readings been below 70 mg/dl with symptoms?	No último mês, com que frequência você teve medidas de glicose menores do que 70mg/dl com sintomas?
6) How often in the last month have you had readings <70 mg/dl without any symptoms?	Com que frequência, no último mês, você teve leituras menores do que 70mg/dl sem sintomas?	Com que frequência, no último mês, você teve leituras menores do que 70mg/dl sem sintomas?	No último mês, com que frequência você teve medidas de glicose menores que 70mg/dl sem sintomas?	In the last month, how often have your glucose readings been below 70 mg/dl without symptoms?	No último mês, com que frequência você teve medidas de glicose menores que 70mg/dl sem sintomas?
7) How low does your blood sugar need to go before you feel symptoms?	Quão baixo seu açúcar no sangue vai antes que você sinta sintomas?	A quanto desce seu açúcar no sangue antes que você tenha sintomas?	Quão baixo precisa ficar o seu açúcar no sangue para você ter sintomas?	How low does your blood sugar level need to go before you experience symptoms?	Quão baixo precisa ficar o seu açúcar no sangue para você ter sintomas?
8) To what extent can you tell by your symptoms that your blood sugar is low?	Até que ponto você pode dizer pelos seus sintomas que o seu açúcar no sangue está baixo?	Em que extensão você pode dizer, pelos sintomas, que seuee açucar está baixo?	Até que ponto você consegue dizer pelos seus sintomas que o seu açúcar no sangue está baixo?	To what extent can you tell, according to your symptoms, that your blood sugar level is low?	Com que frequência você consegue dizer, pelos seus sintomas, que o seu açúcar no sangue está baixo?

Gold Method (Hypoglycemia Perception Scale)					
Original	**Translation 1**	**Translation 2**	**Reconciliation**	**Back Translation**	**Final**
Do you know when your hypos are comencing? Plesase circle one number	Você sabe quando as suas hipoglicemias estão começando? Por favor, circule um número	Você nota quando as suas hipoglicemias estão começando? Por favor, circule um número	Você sabe quando está começando a ficar com hipoglicemia? Por favor, circule um número	Do you perceive when you are starting to get hypoglycemic? Plesase circle one number	Você sabe quando está começando a ficar com hipoglicemia? Por favor, circule um número

Edinburgh Hypoglycemia Scale					
Original	**Translation 1**	**Translation 2**	**Reconciliation**	**Back Translation**	**Final**
Please score the extent to which you experience the following symptoms during a typical hypoglycemic episode (circle one value for each symptom):	Por favor, pontue a extensão com a qual você experimenta os seguintes sintomas durante um episódio típico de hipoglicemia (circule um número para cada sintoma)	Por favor, até que ponto você tem os seguintes sintomas durante um típico episódio de hipoglicemia (circule um número para cada sintoma)	Por favor, pontue a intensidade com a qual você tem os seguintes sintomas durante um episódio típico de hipoglicemia (circule um número para cada sintoma).	Please rate the intensity with which you experience the following symptoms during a typical episode of hypoglycemia (circle one value for each symptom).	Por favor, pontue a intensidade com a qual você tem os seguintes sintomas durante um episódio típico de hipoglicemia (circule um número para cada sintoma).
Confusion	Confusão	Confusão	Confusão	Mental confusion	Confusão mental
Sweating	Sudorese	Sudorese	Suor	Sweating	Suor
Drowsiness	Sonolência	Sonolência	Sonolência	Drowsiness	Sonolência
Difficulty speaking	Dificuldade para falar	Dificuldade de falar	Dificuldade para falar	Difficulty to speak	Dificuldade para falar
Pounding heart	Coração batendo (palpitação)	Palpitação	Palpitações	Palpitation	Palpitações (sensação de coração batendo forte)
Hunger	Fome	Fome	Fome	Hunger	Fome
Nausea	Náusea	Náusea	Náusea	Nausea	Náusea
Trembling	Tremor	Tremores	Tremor	Shivering	Tremor
Headache	Cefaléia	Dor de cabeça	Dor de cabeça	Headache	Dor de cabeça
Odd behaviour	Comportamento estranho	Comportamento estranho	Comportamento estranho	Odd behaviour	Comportamento estranho
Incoordination	Falta de coordenação	Incoordenação	Falta de coordenação	Incoordination	Falta de coordenação

Supplementary Table 1: results of the translations, reconciliation, back translation steps and the final versions of the Clarke and Gold questionnaires and the Edinburgh Hypoglycemia Symptoms Scale

Supplementary Table 2 - Internal consistency of Brazilian Portuguese version of Clarke questionnaire

Question	Cronbach's Alpha	Cronbach's Alpha if Item Deleted
Total	0.804	
1		.765
2		.791
3		.789
4		.790
5-6		.783
7		.768
8		.760

Supplementary table 3: Portuguese version of the: **A)** Clarke questionnaire, **B)** Gold questionnaire and, **C)** Edinburgh Hypoglycemia Symptom Scale.

A) Questionário de Clarke

1) Marque a alternativa que melhor descreve você (marque apenas uma):
   ( ) Eu sempre tenho sintomas quando o meu açúcar no sangue está baixo. (PN)
   ( ) Algumas vezes eu tenho sintomas quando o meu açúcar no sangue está baixo. (PR)
   ( ) Eu não tenho mais sintomas quando meu açúcar no sangue está baixo. (PR)

2) Você deixou de ter alguns dos sintomas que costumava sentir quando seu açúcar no sangue estava baixo?
   ( ) Sim (PR) ( ) Não (PN)

3) Nos últimos seis meses, com que frequência você teve episódios de hipoglicemia (açúcar baixo no sangue) em que tenha se sentido confuso, desorientado ou apático e não conseguiu se tratar sozinho.
   ( ) Nunca (PN)
   ( ) Uma ou duas vezes (PR)
   ( ) Uma vez a cada 2 meses (PR)
   ( ) Uma vez por mês (PR)
   ( ) Mais de uma vez por mês (PR)

4) No último ano, com que frequência você teve episódios de hipoglicemia (açúcar baixo no sangue) em que tenha perdido a consciência (desmaiado) ou tido convulsões, precisando de glicose intravenosa (injeção de glicose na veia) ou glucagon?
   ( ) Nunca (PN)
   ( ) 1 vez (PR)
   ( ) 2 vezes (PR)
   ( ) 3 vezes (PR)
   ( ) 4 vezes (PR)
   ( ) 5 vezes (PR)
   ( ) 6 vezes (PR)
   ( ) 7 vezes (PR)
   ( ) 8 vezes (PR)
   ( ) 9 vezes (PR)
   ( ) 10 vezes (PR)
   ( ) 11 vezes (PR)
   ( ) 12 vezes ou mais (PR)

5) No último mês, com que frequência você teve medidas de glicose menores do que 70mg/dl **com** sintomas?

( )Nunca

( ) 1 a 3 vezes

( ) 1 vez por semana

( ) 2 a 3 vezes por semana

( ) 4 a 5 vezes por semana

( ) Quase diariamente (PR)

6) No último mês, com que frequência você teve medidas de glicose menores que 70mg/dl sem sintomas?

( )Nunca

( ) 1 a 3 vezes

( ) 1 vez por semana

( ) 2 a 3 vezes por semana

( ) 4 a 5 vezes por semana

( ) Quase diariamente (PR)

(Se resposta da 5< que resposta da 6 = PN; se resposta da 5 < que resposta da 6 = PR)

7) Quão baixo precisa ficar o seu açúcar no sangue para você ter sintomas?

( ) 60-69 mg/dL (PN)

( ) 50-59 mg/dL (PR)

( ) 40-49 mg/dL (PR)

( ) Menores de 40 mg/dL (PR)

8) Com que frequência você consegue dizer, pelos seus sintomas, que o seu açúcar no sangue está baixo?

( ) Nunca (PR)

( ) Raramente (PR)

( ) Algumas vezes (PR)

( ) Frequentemente (PN)

( ) Sempre (PN)

---

3 ou menos respostas PN = percepção normal à hipoglicemia,

4 ou mais resposta PR = percepção reduzida à hipoglicemia.

B) Questionário de Gold

Você sabe quando está começando a ficar com hipoglicemia (açúcar baixo no sangue)?

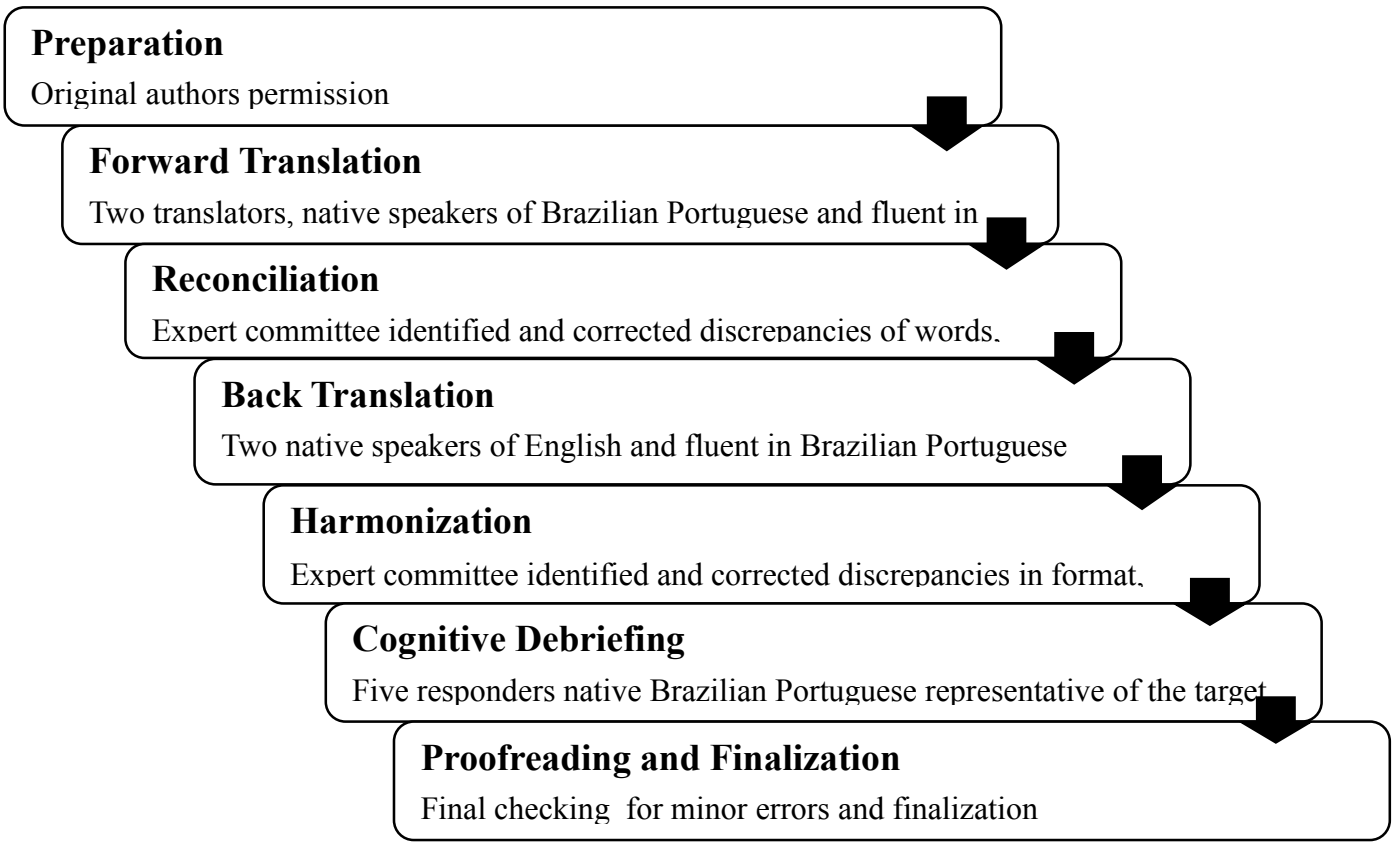
(Por favor circule um número)

Sempre Percebe						Nunca Percebe
1	**2**	**3**	**4**	**5**	**6**	**7**

C) Escala de Hipoglicemia de Edinburgh

Por favor, pontue a intensidade com a qual você tem os seguintes sintomas durante um episódio típico de hipoglicemia (circule um número para cada sintoma).

	Não tenho este sintoma						Tenho este sintoma muito forte
Confusão mental	1	2	3	4	5	6	7
Suor	1	2	3	4	5	6	7
Sonolência	1	2	3	4	5	6	7
Dificuldade para falar	1	2	3	4	5	6	7
Palpitações (sensação do coração batendo forte)	1	2	3	4	5	6	7
Fome	1	2	3	4	5	6	7
Náusea	1	2	3	4	5	6	7
Tremor	1	2	3	4	5	6	7
Dor de cabeça	1	2	3	4	5	6	7
Comportamento estranho	1	2	3	4	5	6	7
Falta de Coordenação	1	2	3	4	5	6	7



Supplementary figure 1 – Diagram of the Translation and Cross-cultural adaptation of the questionnaires

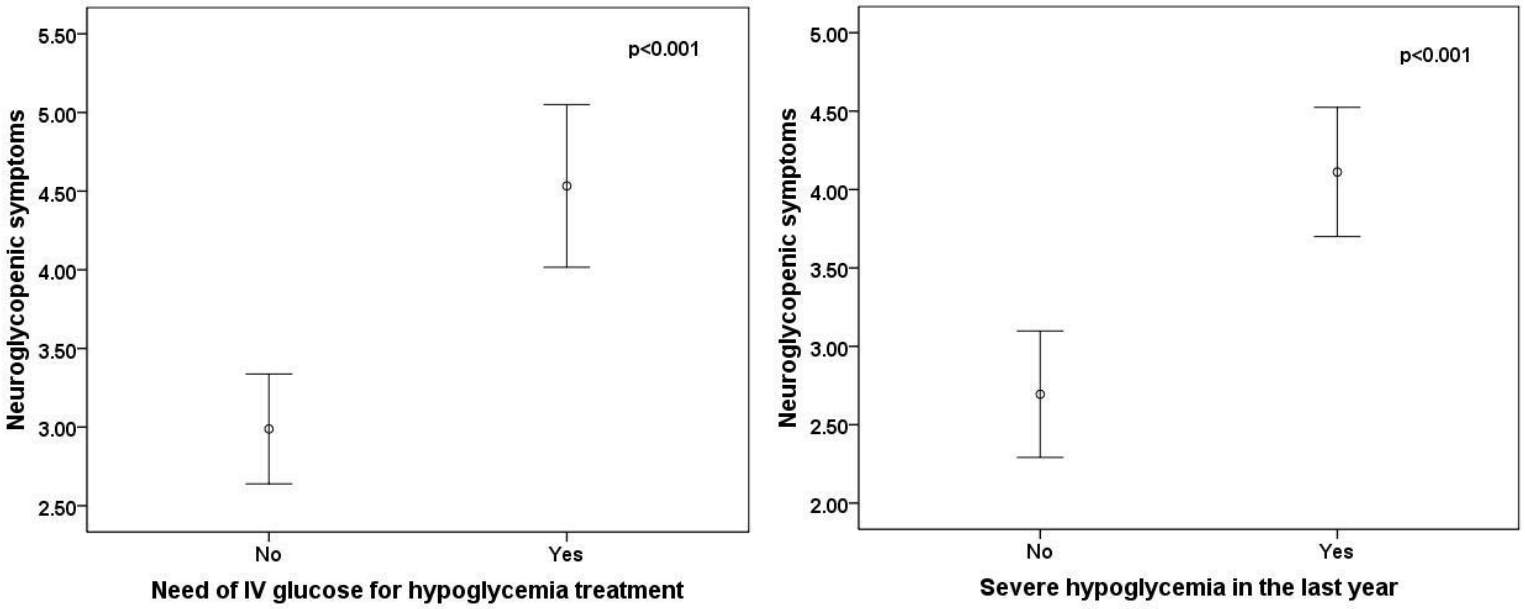


Figure 1 - Neuroglycopenic symptoms subscale of the Edinburgh Hypoglycemia Symptom Scale associated with need of IV glucose for hypoglycemia treatment and with presence of severe hypoglycemia in the last 12 months.

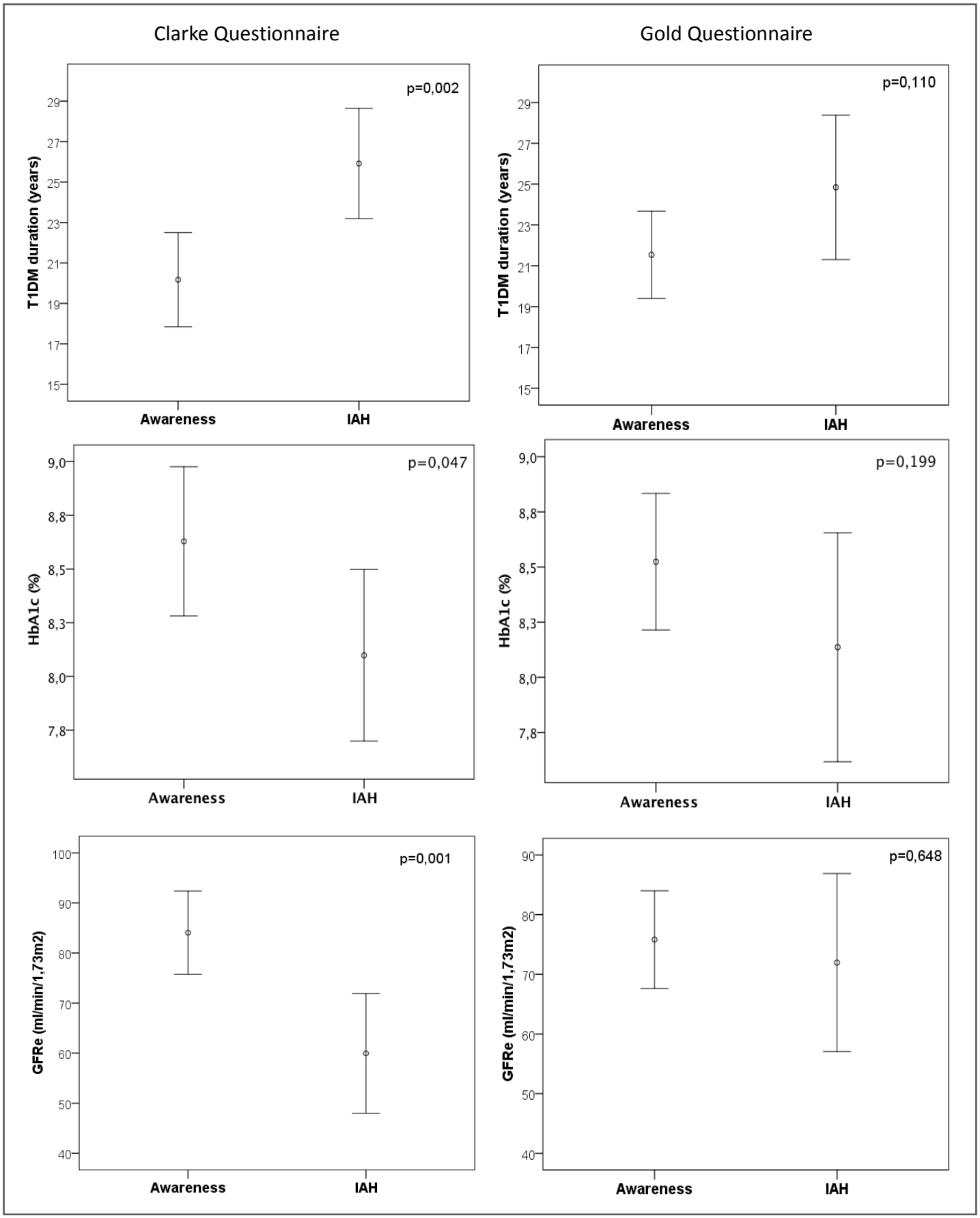


Figure 2: Differences between the Clarke and Gold questionnaires in the ability to detect impaired awareness of hypoglycemia (IAH) according to clinical and laboratory variables, applied in 123 outpatients.

Capítulo 2 – Artigo Original 2

Performance of the flash glucose monitor monitoring system in type 1 diabetes and chronic kidney disease candidates for β-cell replacement therapy.

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Performance of the flash glucose monitor monitoring system in type 1 diabetes and chronic kidney disease candidates for β-cell replacement therapy.

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ABSTRACT

Aims: To evaluate the performance of an interstitial glucose monitoring system (flashGM) compared with the self-monitoring glucose monitoring (SMBG) in candidates for β-cell replacement therapy with type 1 diabetes mellitus (T1DM) and chronic kidney disease (CKD).

Methods: Candidates for beta-cell replacement therapy were recruited and answered a standard questionnaire to collect demographic information. They also answered the Clarke questionnaires to evaluate hypoglycemia awareness, underwent to physical and laboratory evaluations, had the flashGM sensor applied to the back upper arm with an applicator and were instructed to perform and record the SMBG measurements seven times a day including pre-meals, post-meal, and bedtime.

Results: 39 patients were included, with 1420 pairs of matched glucose measurements generated during the 14 day-long flashGM. 41.5% of the patients were on dialysis therapy and the others were post kidney transplant patients; 59% were diagnosed with problematic hypoglycemia and patients checked glucose 2.8 times more frequently with the flashGM than with SBGM. FlashGM values correlated with SMBG ($r = 0.818$, $p<0.001$) and Bland-Altman analysis showed a mean between-method difference in glucose levels of 2.32 mg/dL; 95% limits of agreement between the two methods of glucose measurement were ranging from –122 to 127 mg/dL. The MARD between flashGM and SMBG was 25.28% (95% CI 23.6-27.5%) and most of the paired glucose measurements were in the acceptable accuracy zone (zones A and B), 92.75% for Clarke Error Grid and 94.75% for Consensus Error Grid (CEG) Analysis.

Conclusions: In patients with high glycemic variability and CKD, the use of flashGM may be an additional tool for glycemic monitoring, since it improves the adherence of glycemic monitoring. However, due to the lower accuracy of the flashGM during rapid glycemic excursions, it should be used associated with SMBG to guide therapeutic interventions.

Keywords: diabetes mellitus; glucose monitoring; glycemic control; hypoglycemia; islet transplantation; pancreas transplantation

1. Background

Type 1 Diabetes Mellitus (T1DM) intensive glucose treatment is associated with reduction in DM chronic complications (1, 2). However, as a side effect, 3-fold increased risk of hypoglycemia is observed (3). Despite all the advances in T1DM treatment seem in latter decades, such as insulin analogues, insulin delivery methods, and technologies for glucose monitoring, hypoglycemia still represents a major barrier to optimal glycemic control (4).

Hypoglycemia is often a neglected problem and its consequences are not always well evaluated and valued. Severe neuroglycopenia may lead to seizures and even coma (5). Hypoglycemia have also arrhythmogenic effects (6), results in secretion of hormones that promote hypercoagulability and inflammation (7) and may increase the risk of coronary artery disease (8). Recurrent hypoglycemia changes cerebral blood flow and transient ischemic strokes are recognized manifestations of hypoglycemia (9). In addition, the extremes of age are more susceptible to the deleterious effects of hypoglycemia on cognition (10), and it may contribute to the development of dementia in the elderly (11).

Some patients have problematic hypoglycemia, defined as two or more episodes of severe hypoglycemia per year or one episode associated with impaired awareness of hypoglycemia (IAH), excessive glycemic lability, or major hypoglycemia fear and maladaptive behavior because of that (12). For these patients, β cells replacement therapy, through pancreas or islet transplantation, appears to be a treatment option once it restores endogenous insulin secretion and the awareness of hypoglycemic symptoms (13-15).

Candidates for β-cell replacement therapy may have chronic kidney disease (CKD) as a complication of diabetes and CKD *per si* is an independent risk factor for hypoglycemia, representing an additional risk in such subjects (16-18). The kidney plays an important role in regulation of glucose and insulin homeostasis, by preforming gluconeogenesis and tubular glucose reabsorption (19-21). Patients with moderate to severe CKD have a reduced ability to maintain glycemic homeostasis (22, 23). Altogether, these factors contribute to the difficult task of achieving adequate glucose control in this population.

Evaluation of glycemic control in candidates for β cells replacement therapy is challenging. HbA1c is widely used, and it is considered the standard method to access glycemic status and risk of diabetic complications. However, several clinical situations may falsely interfere with its values (24). One of those is the already mentioned complication of diabetes, CKD, which is associated with significant changes in

erythrocytes turnover caused by anemia, uremia, and erythropoietin analogs use. All these factors interfere with the results of the HbA1c tests, leading to false interpretations (25).

Capillary blood glucose, obtained by self-monitoring of blood glucose (SMBG), is another way to monitor glucose values and is an essential component of intensive T1DM treatment. International guidelines recommend T1DM patients to perform SMBG four to ten times a day, including before each meal, snacks and at bedtime (26, 27). However, many patients fail to adhere to this recommendation (28, 29).

Monitors of interstitial glucose are also available (30) and, most recently, an intermittently scanned continuous glucose monitor, also known as flash glucose monitor (FlashGM) was produced. It is associated with reduction in the incidence and time spent in hypoglycemia and enhanced the adherence of glucose measurements in T1DM patients (31, 32). However, this method has not been tested in T1DM patients with high glycemic lability and chronic kidney disease.

2. Subjects, Material, and Methods

This study was carried out in a diabetes outpatient clinic of a tertiary hospital in Brazil. The patients were recruited between April/2017 and December/2018 and were candidates for beta-cell replacement therapy according to the institutional protocol. Eligibility criteria included: T1DM patients either in dialysis therapy, which are candidates for simultaneous kidney-pancreas transplantation, or renal transplant patients candidates for islet transplantation; aged between 18 and 65 years, had at least one episode of severe hypoglycemia in the last 6 months or inadequate glycemic control (HbA1c >7.5%). Exclusion criteria included patients with active infection, patients that received blood transfusions in the last three months prior to the beginning of the study or who had the dose of erythropoietin analogues modified in the last 30 days.

At the first visit, after obtaining written and verbal informed consent, a standard questionnaire was applied to collect information on age, gender, ethnicity, T1DM duration, medications on use, and presence of diabetic chronic complications. They also answered the Clarke questionnaires to evaluate hypoglycemia awareness (previously validated to Portuguese by our group) (33). All patients underwent physical and laboratory evaluations (fasting plasma glucose, hemoglobin, creatinine, HbA1c). Serum creatinine was measured by the Jaffé reaction and was traceable to IDMS; HbA1c were performed using high-performance liquid chromatography (HPLC).

Later on, the flashGM sensor (FreeStyle Libre™, Abbott Diabetes Care, Witney, UK) was applied to the back upper arm. Patients were on motorized for the length of time the sensor was active (14 days). Information on the FGM screen was not masked. Individuals were instructed on how to use the flashGM, and to perform and record the SMBG measurements seven times a day including pre-meals, post-meal, and bedtime. The point-of-care blood glucose meters used were the ones provided by the public system, and are certified by ISO15197 / 2013 (34). Patients received a diary to record capillary (SMBG) and interstitial glycemia (flashGM) during 14 days. At the second visit (after 14 days), the flashGM sensor was withdrawn, and the glycemic diary record was collected for further analysis. The SMBG was considered the reference blood glucose measurement for the analysis. HbA1c estimated average glucose was calculated by the formula 28.7 X A1C – 46.7 = eAG (35).

The study protocol was approved by the Ethic Committees in Research from Hospital de Clínicas de Porto Alegre (GPPG #16-0652).

The glucose monitoring systems and sensors used in this study were not funded by industry.

Data Analyses

Demographic and clinical data were expressed as mean ± SD and median (minimum; maximum values) for continuous variables, and number and percentages were presented for categorical variables. Glucose measurements by SMBG and flashGM were also assessed through MAD and MARD (36) and the outcomes from these readings were superimposed on Clarke and Parkes/Consensus Error Grid (37).

MARD is a simple metric that summarizes the accuracy of all paired glucose measurement points and can be used to compare different systems and even to suggest a threshold for non-adjunctive use of flashGM instead of SMBG (36). An emerging view is that an arbitrary MARD of 10% represents the level of accuracy required for safe use of CGMS readings to make insulin dosing decisions, without the need for an adjunct SMBG reading (38). MAD gives an indication of the tendency of a glucose sensor to read high or low compared with a reference method, whereas the MARD is the relative deviation of a sensor from a reference (39). To help better assessment of these new technologies, the Clarke error grid was developed (37) and was further refined to the more streamlined Parkes or Consensus error grid (CEG) (40). These grids compare glucose readings of the two methods and plots them on a grid, which is divided into zones. This helps to visualize the accuracy as well as the clinical impact of any system errors.

Readings that fall into zones A and B are accepted for making clinical decisions, with the rest of zones showing reduced and clinically questionable accuracy.

The agreement between the two methods was evaluated by Bland-Altman analysis (41). Pearson correlation coefficient between methods was also calculated. Student´s test was applied for paired differences between methods.

Analyses were performed using SPSS (IBM SPSS Statistics for Windows, V.20.0, Armonk, New York, USA: IBM Corp) and the R Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Thirty-nine T1DM patients were included, with 1420 pairs of matched glucose measurements generated during the 14 day-long flashGM. Four patients discontinued SMBG measurements throughout the study, claiming greater comfort, convenience and absence of pain with the measurement of glycemia by flashGM.

Of the 39 included patients, 51.3% were male, mean age was 38.33 ± 8.68 years old, mean HbA1c was 8.25 ± 1.39% (min-max 6.0 – 11.9%), and mean diabetes duration was 27.56 ± 6.73 years. Other clinical and demographic characteristics are described in **Table 1**. Sixteen patients were on dialysis (41.5%) and 23 (61.5%) were post kidney transplant patients with medium eGFR of 63.7 ± 25.15 ml/min/1.73m².

The majority of patients had problematic hypoglycemia, since 59% reported two or more episodes of severe hypoglycemia in the last year. Concerning the IAH evaluated by the Clarke questionnaire, 51.3% of the patients had positive results. The glycemic variability measured by the SD of the SMBG tests was high in this population, where 45.7% of the patients had a SD of glycemic measurements higher than 50 mg/dl.

Patients check glucose more frequently with flashGM than with SBGM **(Figure 1A).** As expected, a higher frequency of glucose check was associated with more hypoglycemia diagnosis, as more glucose measurements were available **(Figure 1B)**.

FlashGM values correlated with SMBG ($r = 0.818$, $p<0.001$) **(Figure 2A)** and with average glucose estimated by the HbA1c ($r = 0.595$, $p<0.01$). FlashGM did not significantly differ from self-measured glucose (170.82 ± 100 vs. 173.15 ± 92.9 mg/dL, $p = 0.169$)

Bland-Altman analysis showed a mean between-method difference in glucose levels of 2.32 mg/dL; 95% limits of agreement between the two methods of glucose measurement were ranging from –122 to 127 mg/dL **(Figure 2B)**.

The MARD between flashGM and SMBG was 25.28% (95% CI 23.6-27.5%), and the MAD was 38.3 mg/dl (95% CI 35.65-40.95). MARD, MAD and MD for different categories of glucose values are shown in **Table 2**. For glucose values between 70 -250 mg/dl, the MARD showed no statistical differences between the two methods, while values below 70 mg/dl and above 250mg/dl were statistically different between methods. FlashGM measures were lower than SMBG measures in 53.52% (n=761), equal in 4.6% (n=66) and higher 41.76% (n=593).

In order to evaluate the effect of the eGFR on the MARD results, a sub-group analysis was performed between patients in dialysis and post-kidney transplant patients. A marginal statistical difference between the two groups was observed (28.48% vs. 23.83% respectively, p=0.057), with worst results in dialytic patients

The Clarke Error Grid and Consensus Error Grid (CEG) Analysis are shown in **Figure 3**. Most of the paired glucose measurements are in the acceptable accuracy zone (zones A and B), 92.75% for Clarke Error Grid and 94.75% for Consensus Error Grid (CEG) Analysis.

Regarding adverse events related to flashGM, one patient (2.46%) had blood at the sensor site when the sensor was removed, and two other patients (5.1%) had visible skin reaction after removal.

4. Discussion

This study aimed to evaluate the accuracy of flashGM in T1DM patients candidates for β-cell replacement therapy who are prone to high glycemic lability and presenting CKD (15).

FlashGM and SMBG showed a strong correlation, which does not necessarily mean good agreement between the two methods. This was better evaluated by the Bland Altman analyses (42), that revealed a small systemic bias, but with wide limits of agreements, showing clinically relevant differences between methods.

Accuracy of the flashGM was also evaluated by MARD that showed to be higher than most of the studies that evaluate flashGM (43-45). When the MARD values were plotted in Clarke and Consensus Error Grid, the majority of the tests were in zone A and B, meaning “clinically acceptable” and “benign errors, clinically acceptable”.

Previous studies, with T2DM being the majority of patients, demonstrated MARD <15% and more than 80% of the paired glucose values in zone A of CEG (44, 46). Ólafsdóttir et al. (43), evaluated T1DM without CKD showed a MARD of 13,2%.

Schierenbeck et al. (47) who evaluated diabetic patients undergoing cardiac surgery, found a worse performance of flashGM, with a MARD of 30.5% when compared to arterial blood glucose. Another study performed in patients in an Intensive Care Unit, comparing flashGM with SBGM, found a MARD of 20% (48).

Our results are more in line with the last two studies cited. In them, study participants were also more likely to exhibit high glycemic variability due to the underlying diseases, which could cause rapid glycemic excursions. In these situations, flashGM is not approved for non-adjunctive insulin dosing, and blood glucose should be confirmed by the SBGM (39). It occurs because there is a lag time of approximately 4.5 minutes between the plasma and interstitial fluid; therefore, interstitial glucose values do not correspond exactly to blood glucose concentration (44).

It is important to emphasize that the patients on this study had more than 20 years of diabetes duration and had a high prevalence of chronic complications. Because of the difficulties in achieving good glycemic control and or present with high glycaemia variability and complicated hypoglycemia, they were candidates for b-cell replacement therapy. All patients had CKD, 41.5 % were on dialysis and the others had gone through a kidney transplant. Approximately half of the patients had impaired awareness of hypoglycemia, prevalence higher than that described in the literature for T1DM patients in general (49, 50).

In patients with CKD, deficient gluconeogenesis along with malnutrition, inadequate catecholamine release, and impaired renal insulin degradation and clearance contribute to the frequent hypoglycemia episodes (51, 52). On the other hand, hyperglycemia episodes can also be more frequent in patients with CKD, especially on those in dialytic therapy, since uremia affects peripheral tissue sensitivity increasing insulin resistance and the dialytic therapies usually use glucose in dialytic solutions. (53).

Another significant finding, also reported by other studies (43, 46), is the fact that flashGM showed a tendency to measure lower glucose values than SMBG, especially in lower glucose ranges. It is still unclear the mechanisms behind this finding, but one possible explanation is that glucose is transferred from the capillary endothelium to the interstitial fluid by simple diffusion through a concentration gradient and at lower glucose levels the rate of glucose diffusion would be reduced, leading to reading of lower values (54). Regardless of the mechanism, this finding warns of the need for another glucose measurement technique in cases of low glycemic levels measured by flashGM, before any therapeutic actions are initiated.

Despite the limitations observed with the use of flashGM on this population, mainly in the extreme values, the use of this new technology provided a better adherence of patients to glucose monitoring, since they performed 2.8 times more measurements than with the SBGM. This fact is relevant and may impact patient´s health since higher frequency of glucose measurements are associated with better glycemic control (lower HbA1c) (55).

FlashGM also allowed the detection of more episodes of hypoglycemia. In this population, it could mean saving lives, since more than half of them suffer with IAH. These patients exhibit a nearly six-fold higher frequency of severe hypoglycemia than patients without IAH (56) and these episodes are more related to complications such as loss of consciousness, seizures, coma, or death (57).

The study has some limitations. The smaller number of SMBGs performed by the patients than requested may have influenced the analysis. This may have occurred, in part, because of the high prevalence of diabetic retinopathy and blindness in these patients, who very often rely on third parties to perform SMBG. Another possible reason is the long time duration of diabetes (~27 years) and the lack of adherence and belief in new therapeutic strategies.

To our knowledge, this is the first study to evaluate flashGM accuracy in patients with high glycemic variability and CKD or post kidney transplant, candidates for β-cell replacement therapy.

5. Conclusion

In patients with high glycemic variability and CKD, such as T1DM patients candidates for B-cell replacement therapy, the use of flashGM may be an additional tool for glycemic monitoring, since it increases the frequency of glycemic measurements by the patients. However, due to the lower accuracy of the flashGM during rapid glycemic excursions, especially in values lower than 70 mg/dL, it should not be used alone to guide therapeutic interventions. In these cases, the SMBG should be performed to confirm the results. Studies with plasma blood glucose measurements as the reference method must be done to better clarify the use of interstitial glucose measurements in patients with high glycemic variability.

Author Contributions and Acknowledgements

PS, CBL and ACB designed the study, interpreted the data, and reviewed the manuscript. PS and ALMS acquired the data. PS and ACB analyzed the data and drafted the manuscript. All authors approved the final version.

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Declaration of interest

The authors declare no conflict of interest.

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Table 1 - Clinical and Demographical Characteristics of the patients (n=39)

Age	38.33 ± 8.68 36 (27; 59)
Sex (male)	20 (51.3%)
Diabetes Duration (years)	27.56 ± 6.73 27 (14; 42)
BMI (kg/m²)	23.32 ± 3.35 23.02 (17.60; 31.56)
HbA1c	8.22 ± 1.37% 8.05(6.0%; 11.9%)
Insulin dose	0.8 ± 0.18 UI/kg/day 0.82 (0.43; 1.14)
Dialysis	16 (41%)
Kidney transplant eGRF(ml/min/1.73m²)	23 (61.5%) 63.7 ± 25.15
C-peptide	0.03 ± 0.06 0.010 (0.01; 0.3)
Hemoglobin (mg/dL)	12.75 ±2.53 13.25 (8.0; 19.3)
IAH (Clarke questionnaire)	20 (51.3%)
Hypertension	29 (74.4%)
Retinopathy	36 (92.3%)
Peripheral symmetric polyneuropathy	17 (43.6%)

Variables are described as mean (SD) or percentage (%).

Table 2. MARD, MAD and MD for different categories of glucose values.

SMBG Values (mg/dl)	MARD (%)	MAD	MD	n	p
<70	40.46 (26.6 to 54.31)	19.9 (14.02 to 25.78)	-6.67 (-13.32 to -0.209)	143	0.049
70-180	26.85 (23.74 to 29.96)	32.45 (28.99 to 35.9)	-3.88 (-8.06 to 0.299)	723	0.069
180-250	20.62 (18.18 to 23.07)	43.33 (38.2 to 48.45)	-4.51 (-11.67 to 2.63)	290	0.215
>250	17.85 (15.57 to 20.15)	58.77 (50.87 to 66.66)	31.71 (21.8 to 41.63)	264	0.000

Variables are shown in mean (CI 95%)

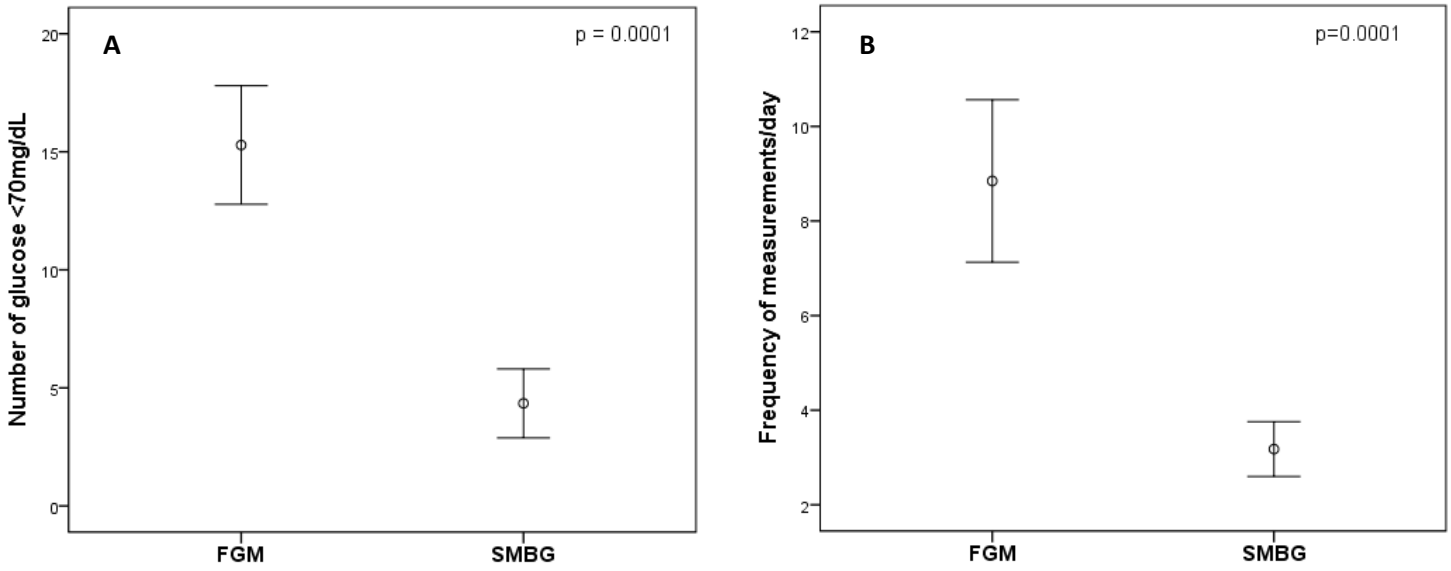


Figure 1. Frequency of glucose monitoring **(A).** Number of hypoglycemias/day **(B).**

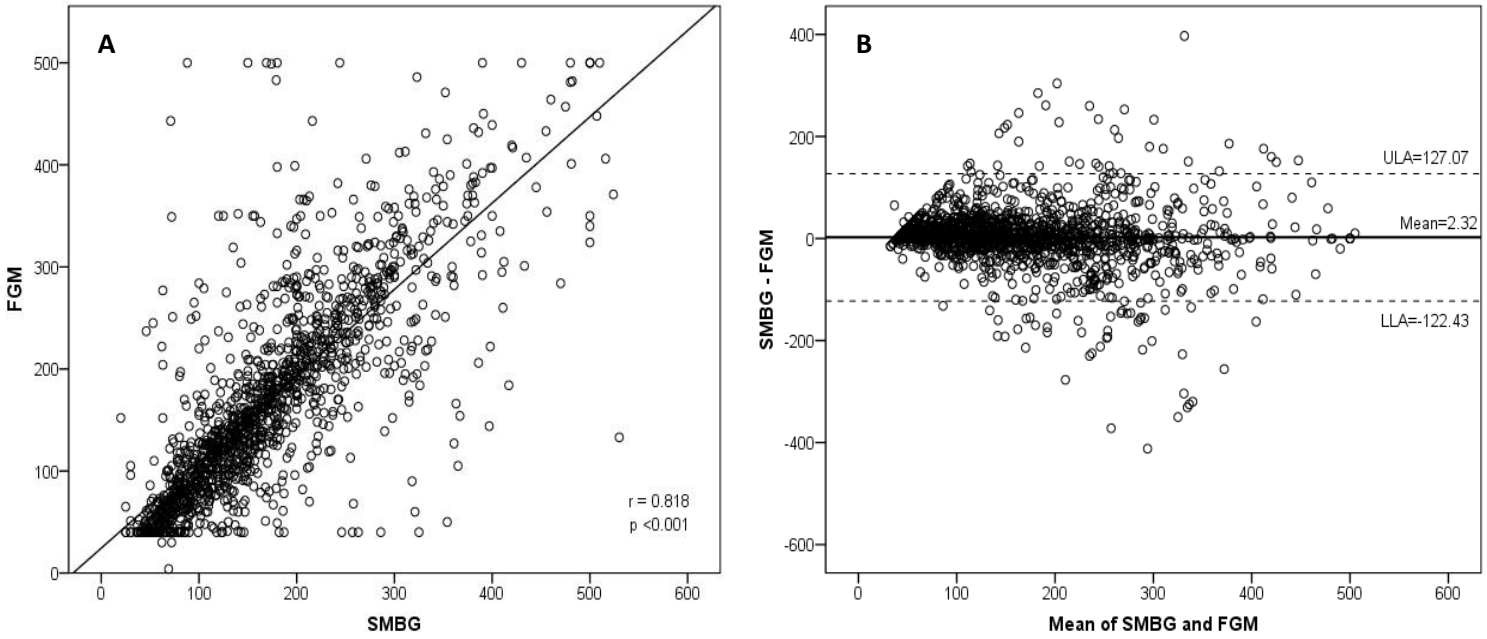
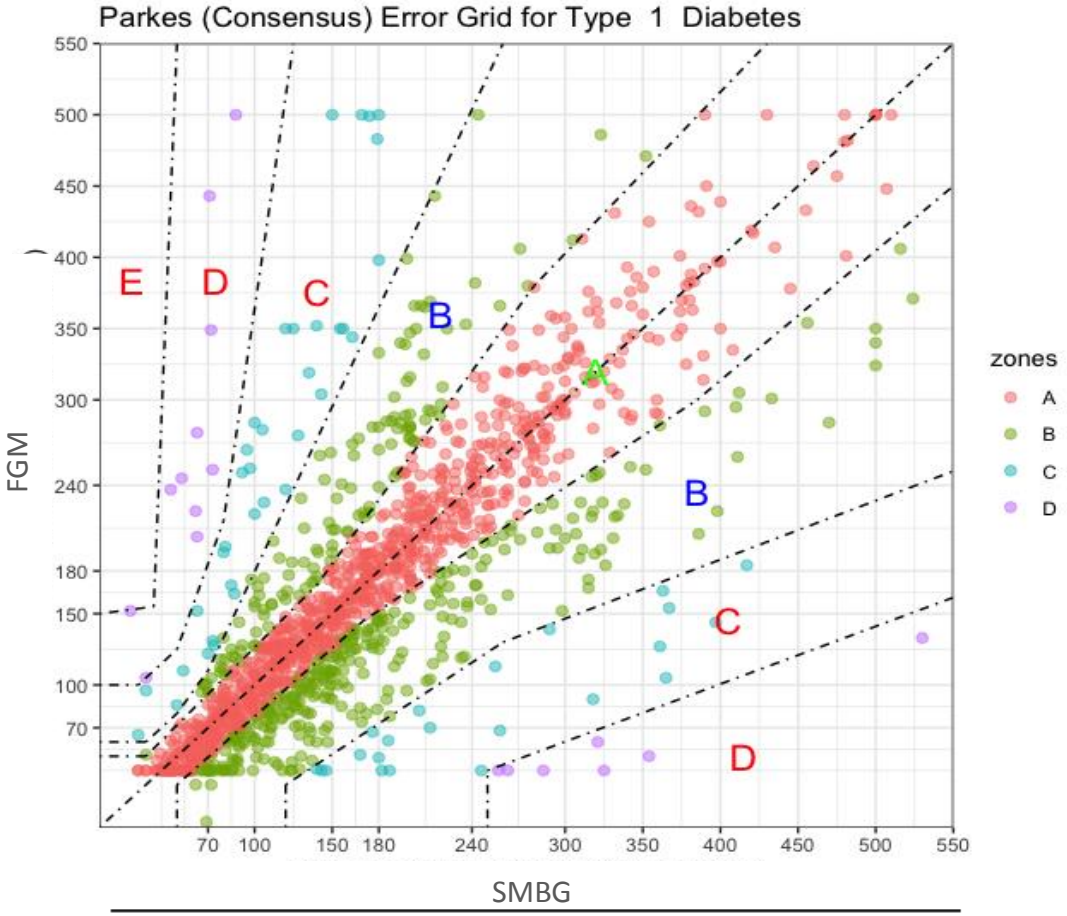


Figure 2. Correlation between SMBG and FGM **(A)**. Bland-Altman analysis between SMBG and FGM **(B)**.

Zone	A	B	C	D	E
Paired glucose	897	420	54	31	18
%	63.17	29.58	3.8	2.18	1.27



Zone	A	B	C	D
Paired glucose	930	415	57	18
%	65,49	29,23	4,01	1,27

Figure 3. Clarke Error Grid and Consensus Error Grid (CEG) Analysis

Capítulo 3 – Considerações finais

A alta prevalência de percepção reduzida a hipoglicemia entre indivíduos com DM1 no nosso meio chama a atenção para a necessidade de maior vigilância em relação os episódios hipoglicêmicos e melhor educação desses pacientes, a fim de reduzir as taxas de morbimortalidade relacionadas a hipoglicemia. A utilização dos questionários validados é de grande valia tanto na prática clínica quanto no âmbito da pesquisa clínica. Os questionários irão auxiliar na identificação dos pacientes em risco para hipoglicemias problemáticas, permitindo ajustar o tratamento insulínico ou avaliar aqueles que são candidatos a terapia de substituição de célula β.

Em pacientes candidatos à terapia de reposição de células β, o uso de flashGM pode ser uma ferramenta adicional para o monitoramento glicêmico. Essa tecnologia aumenta a frequência de medidas glicêmicas pelos pacientes, possibilitando que mais episódios de hipoglicemias assintomáticas, tão comuns nessa população, sejam diagnosticados e tratados precocemente. Entretanto, especialmente nos extremos de glicemia, o sistema flashGM não deveria ser utilizado isoladamente para orientar as intervenções terapêuticas devido à sua menor precisão durante as rápidas excursões glicêmicas, como observado neste estudo. Nestes casos, o SMBG deve ser realizado para confirmar os resultados.

Apresentamos nesta dissertação dois artigos originais que acreditamos terem contribuído de forma significativa para a geração de conhecimento e aplicabilidade clínica.