ORIGINAL ARTICLE

Effect of low-frequency blood glucose self-monitoring on glycosylated hemoglobin levels among older adults with type 2 diabetes mellitus

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Background: Reducing the frequency of self-monitoring of blood sugar, due to needle phobia, pain, stress, and costs associated with the procedure, can improve patient compliance and quality of life, provided that adequate blood sugar control is maintained. This study aimed to evaluate the effect of low-frequency blood glucose self-monitoring (LFBGSM) on glycosylated hemoglobin ($HbA1_C$) levels among older adults living with type 2 diabetes mellitus (T2DM), treated with or without insulin. **Materials and Methods:** This randomized controlled trial with a parallel design was conducted on 121 older adults with T2DM in Sabzevar, Iran, between 2018 and 2020. Initially, subjects were stratified based on the type of treatment (with or without insulin) and then randomly assigned to intervention (LFBGSM) and control (no blood glucose self-monitoring [no-BGSM]) groups. $HbA1_C$ levels were measured at the beginning of the study and 3 months later for all study groups. **Results:** The mean age of participants treated with and without insulin was 64.3 ± 9.60 and 64.7 ± 5.01 years, respectively. The ANCOVA test revealed a significant difference in the mean $HbA1_C$ levels among the four groups 3 months postintervention ($P < 0.001$). The $HbA1_C$ scores significantly decreased in the LFBGSM groups and increased in the no-BGSM groups at 3 months postintervention (insulin/LFBGSM, insulin/no-BGSM, noninsulin/LFBGSM, and noninsulin/no-BGSM: 7.74 ± 0.76, 8.34 ± 1.53, 7.70 ± 0.75, and 8.14 ± 1.11, respectively) compared to baseline (8.25 ± 0.67, 8.03 ± 0.64, 8.08 ± 0.69, and 7.83 ± 0.74, respectively). The least significant difference *post hoc* tests showed significant differences between specific groups, emphasizing subtle responses to interventions (*P* values ranging from 0.001 to 0.929). **Conclusion:** Findings suggest a significant reduction in $HbA1_C$ scores within the LFBGSM groups, while a discernible increase is observed in the no-BGSM groups over the 3 months. These findings underscore the efficacy of the interventions and emphasize the crucial role of personalized approaches in optimizing glycemic control for individuals with diabetes.

Key words: Blood glucose self-monitoring, diabetes mellitus type 2, glycated hemoglobin A, insulin

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INTRODUCTION

Diabetes is one of the most prevalent chronic diseases among older adults.[1] According to the International Diabetes Federation, 415 million adults between 20 and 79 years of age were living with diabetes mellitus in 2015, and this number is projected to reach 642 million adults by 2040.[2] Approximately 50% to 80% of adults aged 65 years and older have impaired glucose tolerance.[2,3] In 2015, 13% of Iranian adults were affected by diabetes. Iran has the third-fastest growing diabetes

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epidemic in North Africa and the Middle East, after Egypt and Pakistan.[4] Globally, a person dies every 10 s due to inadequate awareness of diabetes control. Therefore, emphasizing self-care education should be a priority for effective diabetes management.[4]

Physiologic changes in older adults can result in difficulties in diagnosing and treating diabetes as symptoms may be difficult to detect. The absence of symptoms associated with blood sugar fluctuations in older adults contributes to the risk of complications, such as cardiovascular diseases,[5] amputations, visual impairment, and nephropathy.[2] The Framingham study conducted in 2010 also revealed that people with diabetes over 50 years old live on average 7.5–8.2 fewer years than people without diabetes. Therefore, early diagnosis of diabetes and prompt initiation of treatment and monitoring are of utmost importance.[6]

The strategy of blood glucose self-monitoring (BGSM) has been introduced to control blood glucose levels, which can be effective in treating people living with diabetes and preventing diabetes among people at high risk of developing the disease.[1,7] Modifications can be made to adjust diets, physical activities, timely referrals to physicians, and possibly changes in lifestyle to minimize complications.[1,7] Proper blood glucose control can prevent many complications associated with diabetes.[8] It has been estimated that a 1% decrease in glycosylated hemoglobin ($HbA1_C$) levels can reduce 37% of complications such as retinopathy, nephropathy, and neuropathy as well as 21% of the incidence rates of stroke and coronary artery diseases.[8] However, adherence to BGSM has been reported to be poor, with as many as 60% of patients with type 1 diabetes and 67% of patients with type 2 diabetes performing BGSM less frequently than recommended.[9] Several barriers to adherence have been reported, including the high cost of strips and needles, fear of high blood sugar results, stigma, needle phobia, pain, inconvenience, lack of motivation, unconducive workplace, and lack of knowledge as well as self-efficacy to implement BGSM.[10-12] Even in Sweden, where glucometers and strips are provided free of charge, more than 50% of patients with type 1 diabetes do not follow the recommended BGSM more than 4 times a day, citing pain and discomfort caused by lancets as one of the reasons for noncompliance.[13] Therefore, reducing the number of BGSM visits with proper control of patients' blood sugar may increase patients' adherence to this treatment.

Despite the evidence, the value and frequency of BGSM in patients with type 2 diabetes mellitus (T2DM) remain a matter of debate.[14,15] High-frequency BGSM, even once daily, has been associated with symptoms such as anxiety, distress, and depression. However, some studies have shown that high-frequency BGSM is better at controlling blood sugar in people who can adjust their insulin dose.[16] Low-frequency BGSM (LFBGSM) can minimize diabetes-related health-care costs.[17] The treatment of diabetes, with or without insulin, is a factor that influences recommendations for performing BGSM.[17,18]

As there were no studies reported in the literature that compared the effect of LFBGSM between patients treated with and without insulin, this study aimed to evaluate the effect of LFBGSM on $HbA1_C$ levels in older adults with T2DM who were treated with and without insulin.

METHODS

This randomized controlled trial with a parallel design was conducted between 2018 and 2020 to assess the effect of LFBGSM on $HbA1_C$ levels in older adults with T2DM who were treated with and without insulin.

Participants

The study population included all patients with T2DM referred to the diabetes clinic of Sabzevar, Iran, between June 5, 2018, and May 5, 2020. The inclusion criteria consisted of patients with Stage 2 T2DM who were eligible for inclusion in the study if they had regular monthly visits during the study, $HbA1_C$ levels of 6.5%–9%, and a lack of ketones in two first-morning urine samples. Disease severity was measured using the four-stage model for T2DM: Stage 1, $HbA1_C$ 5.7%–6.5%; Stage 2, $HbA1_C$ 6.5%–9.0%; Stage 3, $HbA1_C$ above 9.0%; and Stage 4, $HbA1_C$ of 12% and/or diabetic emergencies.[19] The ability to produce and use insulin is more impaired in Stage 2 compared to Stage 1, and complications are more common, especially in the circulatory and nervous systems. Moreover, metabolic syndrome is frequently observed in Stage 2. Patients were excluded from the study if they had major depression, Cushing's disease, sickle cell disease, glucose 6 phosphate dehydrogenase deficiency, were in the second or third trimesters of pregnancy or the postpartum period, were receiving hemodialysis, had human immunodeficiency virus, had unreliable results (falsely elevated or lowered $HbA1_C$ levels) due to certain conditions, including hemoglobinopathies, anemia, lead poisoning, chronic alcoholism, or opioid use, had experienced recent blood loss or transfusion, were receiving erythropoietin therapy, were unable to follow-up with trial procedures, or were unable to perform BGSM.

Interventions

After the assessment visit and confirmation of eligibility, patients were stratified into two main groups: those treated with insulin and those treated without insulin. Within these groups, patients were then assigned to one of two

subgroups: those who performed LFBGSM and those who did not perform BGSM. The intervention began at the first visit after randomization and ended at the scheduled visit after 3 months. The intervention group was allotted to LFBGSM. Patients performing LFBGSM were asked to record four blood sugar values daily for 3 days per week (2 working days and 1 weekend day) within 12 weeks using a blood glucose meter.[14] Participants were instructed to monitor their blood glucose levels by fasting (e.g., after waking up), before lunch, before dinner, and before bedtime. The target blood glucose levels after fasting and before meals were 4–6 mmol/L and 6–8 mmol/L 2 h after meals. Participants were informed to contact their doctor if their blood glucose results were continually high (>15 mmol/L) or low (<4 mmol/L). They were also instructed on the interpretation of their blood glucose readings. This training was performed by an endocrinologist and a master's degree student in geriatric nursing before the start of the study. During the study, patients' questions were answered by these two individuals through telephone calls. The control group participants with no-BGSM were responsible for monitoring and regulating their own insulin levels according to their usual routine, without any specific instructions from the research team. It should be noted that participants in this group were followed up by the researcher and instructed when they were potentially at risk for adverse health outcomes. To help ensure their safety, the investigator in charge of the study asked participants about symptoms commonly associated with hypoglycemia or hyperglycemia, such as dizziness, confusion, or excessive thirst. In addition, the investigator provided participants with educational materials and counseling on how to recognize and respond to symptoms of hypo- or hyperglycemia. The National Institute for Health and Care Excellence (NICE) guidance was used to select the LFBGSM. The recommendation is 0–7 times self-monitoring of blood glucose (SMBG) in T2DM without insulin therapy and 4–28 times SMBG in T2DM with insulin therapy. Therefore, four times a week was selected to share the same time between the two groups and allow comparison between them.[14] To ensure the accuracy of LFBGSM results, the researcher nurses had weekly telephone calls with both subgroups and obtained the amounts of blood sugar registered by the subjects and their main caregivers for the 3 months of the study. To avoid contamination in the control group, the following measures were taken: (i) asking subjects not to share the contents of the intervention with others; (ii) reducing waiting time while taking $HbA1_C$ samples so that participants did not meet in the waiting room; and (iii) avoiding the transfer of participants among clinicians. Each subject was given a new easy-to-use MICROLET®NEXT lancing device from Ascensia to measure their blood glucose. This device is calibrated, coded, and approved by the Diabetes Technology Society, and its results are derived from the Blood Glucose Monitor System Surveillance Program.[20]

Intervention delivery

The nurses involved in the research study received training and support to ensure patient compliance with the study protocol.[21] The nurses were, respectively, taught and trained in techniques and skills, including psychological theory and behavior change, to administer interventions that included 4 days of case-based training over 4 weeks. The intervention protocols included scripts of different topics that should be used to guide nurses when communicating with patients. Additional measures, including evaluation of sessions recorded by the nurses, were taken. Moreover, an external investigation by a sociologist was conducted to ensure compliance with the intervention protocols. Instructions were outlined in the patient diaries to assist patients in promoting adherence to their specific interventions.[22]

Outcome

The primary endpoint of this study was to determine the $HbA1_C$ levels in the study groups at the beginning and end of the study (3 months later). $HbA1_C$ levels were categorized into three levels, including normal (levels below 5.7%), prediabetes (levels between 5.7% and 6.4%), and diabetes (levels of 6.5% or greater). Approximately 2–3 cc venous blood samples were drawn in the morning (9–10 am) from each participant at baseline and 3 months later to assess the $HbA1_C$ levels. The blood samples were analyzed using an $HbA1_C$ enzyme-linked immunosorbent assay kit in the laboratory.

Sample size determination

Sample size determination was derived from previous trials involving T2DM patients. The study population comprised 150 individuals, and the parameters considered were as follows: 90% power, an effect size of 0.56 (pertaining to the desired outcome, $HbA1_C$ levels), a two-sided significance level of 0.05, and a 10% dropout rate.

Randomization and allocation concealment

Randomization and allocation concealment were performed using a computer-generated random list prepared by a statistician from the Clinical Research Management Organization for the study. The randomization codes were anonymous to researchers at the clinical site. Randomization was stratified by diabetic treatment regimens of participants at the time of registration (insulin and without insulin regimens). Random permuted blocks of size 4 were applied for each stratum. Patients were assigned to each stratum with a ratio of 1:1. Each stratum was provided with 75 sealed, sequentially numbered, opaque envelopes containing group assignments, provided by the clinical trial management organization's staff. Researchers were instructed to write

the number from 1 to 75 on the envelopes, shuffle them, and distribute them to the patients in each stratum. Finally, the envelope was opened, and the treatment assigned to the patient was discussed.

Ethical considerations

Ethical considerations were taken into account, and the study was approved and registered by the Regional Ethics Committee of Sabzevar University of Medical Sciences and the Iranian Registry of Clinical Trials (IRCT) with the code numbers of IR.MEDSAB.REC.1396.118 and IRCT2017225038055N1, respectively. All stages of the study were explained to the participants, and written informed consent was obtained. Each subject was assigned a unique identification code, with all data remaining confidential and anonymous.

Statistical analysis

Statistical analysis was performed by summarizing continuous and categorical variables as mean (standard deviation) and frequency (%), respectively. After checking the normality of variables using the Kolmogorov–Smirnov test (K-S test or KS test), the baseline data were compared using the independent *t*-test and Chi-squared tests or nonparametric equivalent as appropriate. A paired *t*-test was used for comparing $HbA1_C$ levels before and after intervention in each group. ANCOVA test was utilized to compare mean $HbA1_C$ levels among groups, with baseline, diabetic foot, and history of hypoglycemia considered as covariates.[23] In addition, to adjust for multiple significance testing in the ANCOVA analysis, we applied a least significant difference (LSD) *post hoc* test. The analysis of the data was conducted using the intention-to-treat approach. All analyses were performed using STATA (version 12, Stata Corp, College Station, Texas, USA). The sample size was determined using the G*Power software version 3.0.10 created by Heinrich-Heine-Universität Düsseldorf in Düsseldorf, North Rhine-Westphalia, Germany.

RESULTS

After assessing eligibility, a total of 150 participants were randomized, and 121 participants completed the study [Figure 1]. The mean age of the patients treated with and without insulin was 64.3 ± 9.60 and 64.7 ± 5.01 years old, respectively. Seventy-eight (64.46%) participants were female. The baseline demographic and clinical characteristics of the participants are presented in Table 1. It

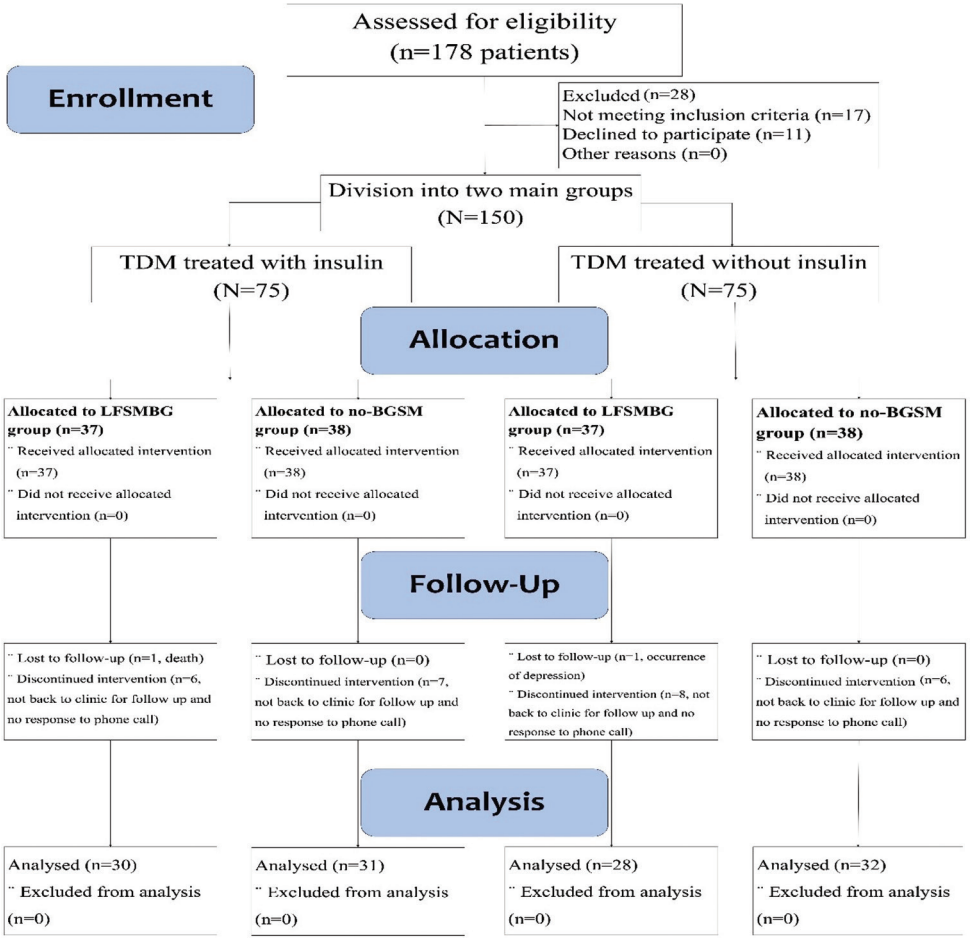

Figure 1: CONSORT diagram of the study

should be noted that the mean $HbA1_C$ levels at baseline and 3 months later were 8.02 ± 0.71 and 7.99 ± 1.11, respectively.

As shown in Table 2, based on the ANCOVA test, the ANCOVA test revealed a statistically significant difference in the mean $HbA1_C$ levels among the four groups 3 months after the intervention ($P < 0.001$). The $HbA1_C$ scores significantly decreased in the LFBGSM groups and increased in the no-BGSM groups at 3 months postintervention (insulin/LFBGSM, insulin/no-BGSM, noninsulin/LFBGSM, and noninsulin/no-BGSM: 7.74 ± 0.76, 8.34 ± 1.53, 7.70 ± 0.75, and 8.14 ± 1.11, respectively) compared to baseline (insulin/LFBGSM, insulin/no-BGSM, noninsulin/LFBGSM, and noninsulin/no-BGSM: 8.25 ± 0.67, 8.03 ± 0.64, 8.08 ± 0.69, and 7.83 ± 0.74, respectively).

Furthermore, the LSD *post hoc* test identified statistically significant differences between the insulin/LFBGSM and insulin/no-BGSM groups ($P = 0.001$), as well as between the noninsulin/LFBGSM and noninsulin/no-BGSM group ($P = 0.006$). In addition, there were significant differences between the insulin/LFBGSM and noninsulin/LFBGSM groups ($P = 0.001$), as well as between the noninsulin/LFBGSM and insulin/no-BGSM groups ($P = 0.008$). However, no statistically significant differences were observed between the insulin/LFBGSM and noninsulin/LFBGSM groups ($P = 0.619$), as well as between the insulin/no-BGSM and noninsulin/no-BGSM group [$P = 0.929$, Table 2].

DISCUSSION

The aim of this study was to assess the effect of LFBGSM on $HbA1_C$ levels in T2DM patients treated with and without insulin. The findings showed that LFBGSM can significantly

Table 1: Baseline demographics and clinical characteristics of patients among groups

Parameters	Groups				*P**
	Treated with insulin		Treated without insulin		
	LFBGSM[a]	No-BGSM[b]	LFBGSM	No-BGSM	
Age (years)	64.2±12.9	64.5±4.72	65.5±5.3	64.3±4.63	0.892
Sex					
Female	19 (63.3)	21 (67.7)	14 (50.0)	24 (75.0)	0.234
Male	11 (36.7)	10 (23.3)	14 (50.0)	8 (25.0)	
Occupation					
Employed	4 (2.3)	2 (6.5)	1 (3.6)	1 (3.1)	0.485
Retired	7 (23.3)	7 (22.6)	11 (39.3)	10 (31.2)	
Homemaker	19 (63.3)	20 (64.5)	16 (57.1)	20 (62.5)	
Unemployed	0	2 (6.5)	0	1 (3.1)	
Household type					
Alone	1 (3.2)	2 (6.5)	1 (3.6)	4 (12.5)	0.415
Living with spouse	27 (90.9)	26 (83.9)	27 (96.4)	27 (84.4)	
Living with children	2 (6.7)	3 (9.7)	0	1 (3.1)	
Marital status					
Single	0	1 (3.2)	1 (3.6)	0	0.202
Married	18 (20.0)	26 (28.8)	21 (23.3)	25 (27.7)	
Deceased/divorced spouse	4 (26.6)	4 (26.6)	0	7 (46.7)	
Educational level					
Illiterate	16 (32.0)	11 (22.0)	10 (20.0)	13 (26.0)	0.909
Elementary	8 (16.6)	13 (27.0)	16 (33.3)	11 (22.9)	
Diploma or above	6 (26.0)	4 (17.3)	5 (21.7)	8 (34.7)	
BMI[c] (kg^2)					
Normal	10 (34.7)	4 (14.2)	8 (28.5)	6 (21.4)	0.759
Overweight	10 (21.2)	13 (27.6)	11 (23.4)	13 (27.6)	
Obese	12 (26.0)	11 (23.9)	12 (26.0)	11 (23.9)	
Hypertension, yes	25 (83.3)	24 (77.4)	19 (67.9)	20 (62.5)	0.254
Cardiovascular diseases, yes	17 (56.7)	16 (51.6)	9 (32.1)	12 (37.5)	0.187
History of severe hypoglycemia, yes	20 (66.7)	21 (67.7)	9 (32.1)	16 (50.0)	0.020
Hospitalization, yes	9 (30.3)	11 (35.5)	7 (25.0)	6 (18.8)	0.492
Diabetic foot ulcers, yes	6 (20.0)	4 (12.9)	7 (25.0)	0	0.030
Retinopathy complications, yes	22 (73.3)	19 (61.3)	13 (46.7)	15 (46.9)	0.106
Nephropathy complications, yes	9 (30.0)	5 (16.1)	6 (15.0)	3 (9.4)	0.207

Mean±SD and *n* (%).*Independent *t*-test and Chi-squared tests or nonparametric equivalent as appropriate, [a]No self-monitoring of blood glucose, [b]Low frequency of self-monitoring of blood glucose, [c]BMI, normal weight (BMI <25), overweight (25≤ BMI <30), and obesity (BMI ≥30). BMI=Body mass index; LFBGSM=Low-frequency blood glucose self-monitoring; No-BGSM=No-blood glucose self-monitoring; SD=Standard deviation

Table 2: Comparisons of hemoglobin glycosylated hemoglobin levels among four groups

Groups	Follow-up			*Post hoc* test	Treated with insulin		Treated without insulin	
	Before intervention (mean±SD)	After intervention (mean±SD)	*P*[e]		LFBGSM (mean difference; *P*)	No-BGSM (mean difference; *P*)	LFBGSM (mean difference; *P*)	No-BGSM (mean difference; *P*)
Treated with insulin				LSD[d]				
LFBGSM[a]	8.26±0.73	7.83±0.79	0.007	*post hoc* test	-	(−0.79; 0.001)	(−0.12; 0.619)	(−0.81; 0.001)
No-BGSM[b]	8.06±0.67	8.41±1.58	0.133		(0.79; 0.001)	-	(0.67; 0.008)	(−0.02; 0.929)
Treated without insulin								
LFBGSM	8.00±0.76	7.75±0.88	0.089		(0.12; 0.619)	(−0.67; 0.008)	-	(−0.69; 0.006)
No-BGSM	7.68±0.78	8.12±1.15	0.028		(0.81; 0.001)	(0.02; 0.929)	(0.69; 0.006)	-
P[c]	0.128	<0.001	-					

[a]Low frequency of self-monitoring of blood glucose, [b]No self-monitoring of blood glucose, [c]ANCOVA test with baseline, diabetic foot, and history of hypoglycemia as covariates, [e]Paired *t*-test, [d]LSD test. LSD=Least significant difference; LFBGSM=Low-frequency blood glucose self-monitoring; No-BGSM=No-blood glucose self-monitoring; SD=Standard deviation

decrease $HbA1_C$ levels in T2DM patients treated with insulin and LFBGSM compared to those treated with insulin and no-BGSM. These results are consistent with the findings of other studies that reported a decrease in $HbA1_C$ levels among insulin-treated diabetic patients.[24,25] SMBG has been defined as an essential way to promote glycemic control in T2DM patients treated with insulin.[26,27] Given the ease of use of SMBG, this approach can reduce frequent referrals of patients to health care and diagnostic centers[7] and also create motivation in terms of adjusting insulin doses in diabetic patients treated with insulin as a strategy to control and decrease $HbA1_C$ levels.[24] Although frequent monitoring of blood glucose (at least once daily) among insulin-treated T2DM patients has been reported to significantly improve $HbA1_C$ levels, low-frequency monitoring has also been reported to be effective.[18] This agrees with the results of the present study. It appears that monitoring in the recommended minimum range (four times a week) as per NICE guidance on insulin therapy for T2DM patients can significantly improve $HbA1_C$ levels.[14]

Results of the present study also showed that LFBGSM can decrease $HbA1_C$ levels in insulin-free T2DM patients compared to those who are insulin-free and not using BGSM. Franciosi *et al*. investigated the association between SMBG frequency and metabolic control and quality of life and noted that high-frequency SMBG is not associated with better control of blood sugar in T2DM patients who do not take insulin. However, high-frequency SMBG is correlated with psychological symptoms such as distress, anxiety, and depressive symptoms.[16] The results of this study revealed that LFBGSM significantly affected $HbA1_C$ levels in two groups of older adult T2DM patients treated with and without insulin. The observed decrease in $HbA1_C$ levels indicates that the impact of LFBGSM on reducing $HbA1_C$ in the insulin-treated group is greater than in the noninsulin-treated group. Other studies have also reported the usefulness of SMBG for insulin-treated T2DM patients and type 1 diabetic patients.[17,27] This is because patients can adjust their insulin dose in accordance with SMBG results.[27] Research questions about the usefulness of SMBG for insulin-free T2DM patients have also been studied.[25]

Limitations and strength

The study has some limitations, including a relatively small sample size and a short duration, which may limit the generalizability of the findings. Further research with larger sample sizes and longer follow-up periods is needed to confirm the study's results. Nonetheless, the study provides valuable insights into the potential usefulness of LFBGSM technology in diabetes management and highlights the importance of regular monitoring of blood glucose levels in promoting glycemic control.

Implications for practice

The psychological side effects of performing high-frequency SMBG, along with increased patient costs and unwillingness to perform SMBG frequently, may reduce patients' adherence to treatment. Determining the minimum number of SMBG required to have a positive therapeutic effect in reducing $HbA1_C$ levels can be beneficial. The results of this study provide helpful insights in this regard.

CONCLUSIONS

The findings suggest a significant and clinically meaningful shift in the mean $HbA1_C$ levels among the four groups 3 months postintervention. Notably, $HbA1_C$ scores exhibited a favorable decrease in the LFBGSM groups, contrasting with an increase in the no-BGSM groups at 3 months. Noteworthy differences were observed between specific intervention pairs, emphasizing the impact of treatment approaches on glycemic control. The insulin/LFBGSM and noninsulin/LFBGSM groups exhibited the most favorable outcomes. Thus, incorporating LFBGSM into multi-factor treatment strategies may be an effective approach for improving glycemic management and preventing the progression of diabetes-related complications. However, caution is warranted when drawing conclusions from the existing literature, as

further research is needed to fully understand the benefits and limitations of LFBGSM. Reducing the frequency of BGSM may increase patients' adherence to the treatment, reduce the cost to patients and the health-care system, and minimize stigma, fear of needles, pain, and discomfort associated with repeated BGSM. In addition, future studies should aim to examine the relationships between LFBGSM and various outcomes in greater detail, to better understand the potential benefits and limitations of this approach.

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Authors' contribution

1. M.S: Design of the work; analysis and interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
2. At.A: Acquisition of data; drafting the manuscript; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
3. F.A: Interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
4. N.R: Conception and design; interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
5. S.J.M: Interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
6. MH.R: Design of the work; analysis and interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
7. Ab.A: Conception and design; interpretation of data; revising manuscript critically; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interest

There are no conflicts of interest.

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