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Glycemic control in type 2 diabetic patients during the COVID-19 virus pandemic

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Abstract

Background: The Coronavirus disease 2019 (COVID-19) disease is a pandemic disease spread worldwide and results in lifestyle changes in areas affected by COVID-19. The ongoing social distancing and lockdowns may negatively impact access to medical care and management of type 2 diabetes mellitus (T2DM). Accordingly, we examined the impact of the COVID-19 virus pandemic in Thailand on the glycemic control of patients with T2DM.

Method: This study focused on T2DM outpatients at Rajavithi Hospital. Three hundred and fifty participants were included. Baseline characteristics, data on exercise, outdoor activities, and access to foods and blood chemistries, including hemoglobin A1C (A1C) and fasting plasma glucose (FPG), were reviewed, and collected from electronic medical records before and after the COVID-19 pandemic.

Results: There was a significant increase in mean A1C (g/L) ± SD (74.8 ± 13.7 vs. 76.0 ± 15.3, p-value <0.016), the mean duration of outdoor activities (hours/day) ± SD during the COVID-19 virus pandemic was significantly decreased. (5.35 ± 4.48 vs. 4.03 ± 4.37, p-value <0.001)

Conclusion: The present study showed that mean A1C was significantly increased during the COVID-19 virus pandemic. Nevertheless, a statistical difference was not observed in FPG. The impact of quarantine, social distancing, and community containment during the epidemic on lifestyles may be the essential factor in increasing A1C.

Keywords: Glycemic control; Hemoglobin A1C; Fasting plasma glucose; Type 2 diabetes mellitus; Coronavirus disease 2019 (COVID-19)

1. Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 was first reported in Wuhan, China, in December 2019 and has spread worldwide. The fatality rate for COVID-19 has been estimated to be 0.5–1.0% [1,2,3]. In Southeast Asian countries, Thailand is the first country that reported a case of COVID-19 outside of China. The first confirmed case in Thailand was announced on January 17, 2020. The highest number of confirmed cases in Thailand was reported on March 22, 2020. There were 188 new cases of tested positive for COVID-19 who related boxing stadium and drinking venue. An immediate nationwide lockdown was instituted in Thailand, which protected Thailand citizens from COVID-19. The government of Thailand announced a nationwide curfew on April 3, 2020 [4]. To stem the transmission of COVID-19 infection, strategies aimed at reducing the frequency and closeness of contact between people are essential [5].

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Diabetes mellitus (DM) is a common chronic disease for which lifestyle modification and medication are needed to control plasma glucose levels. Diabetes seems to be one of the leading causes of mortality and is a public health burden [6]. Glycemic control is of significant importance in DM as complications associated with long-term hyperglycemia are frequent causes of disability and premature mortality and vital drivers of indirect costs. Meanwhile, the persistently elevated blood glucose levels in individuals with DM were considered to increase the predisposition to infectious processes and poor prognosis [7, 8] .

A previous retrospective study at Fujian Provincial Hospital in China was reported that elderly Type 2 diabetes mellitus (T2DM) had a significant increase in fasting plasma glucose (FPG) levels during the COVID-19 pandemic. Nevertheless, a statistical difference was not observed in hemoglobin A1C (A1C), indicating that plasma glucose levels rise short term rather than long-term [9]. The ongoing social distancing and lockdowns may have negatively impacted access to medical care and management of T2DM. Accordingly, we examined the impact of the COVID-19 virus pandemic in Thailand on the glycemic control of patients with T2DM.

2. Material and methods

This study focused on T2DM outpatients at Rajavithi Hospital and this study was approved by Rajavithi Hospital ethic committee number 114/2563. Three hundred and fifty participants were included. Baseline characteristics and blood chemistries, including A1C and FPG, were reviewed and collected from electronic medical records before the COVID-19 pandemic. Between March 22, 2020, and August 22, 2020, which is during the COVID-19 pandemic, patients would be examined by doctors. Bodyweight, blood pressure, and blood chemistries were measured at each visit. Blood chemistries were measured at least two months after baseline. We also collected data on exercise, outdoor activities, and the ability to access foods before and during the COVID-19 pandemic by interviews and questionnaires.

2.1. Inclusion criteria

- Patients with T2DM and aged more than 18 years old
- Willing to participate, interview and do questionnaire. (Informed consent was obtained from all individual participants included in the study)

2.2. Exclusion criteria

- The condition prolongs the erythrocyte life or is associated with decreased red cell turnover, resulting in falsely elevated A1C. Conditions such as Iron deficiency anemia, vitamin B-12, folate deficiency anemias, asplenia, and chronic alcohol drinking.
- The condition that shortens the erythrocyte life or is associated with increased red cell turnover, resulting in falsely lowered A1C. Conditions such as acute and chronic blood loss, hemolytic anemia, thalassemia, and splenomegaly.
- Pregnancy
- Patient with COVID-19 infection

2.3. Sample size calculation

From the literature review, we calculated sample size from two dependent proportions (pre-post proportion)

$$n = \left[\frac{z_{1-\frac{\alpha}{2}}\sqrt{p_{01}+p_{10}}+z_{1-\beta}\sqrt{p_{01}+p_{10}-(p_{01}-p_{10})^2}}{\Delta}\right]^2$$

n = Population size

$Z_{\alpha/2}$ = Critical value of the normal distribution at $\alpha/2$ for a confidence level of 95%, α is a 0.05 and the critical value is 1.96

Z_{β} = Critical value of the normal distribution at β for a power of 80%, β is a 0.2 and the critical value is 0.84

Base on retrospective observational study of Tsubokura, 2013 [10] .

P_1 = Proportions of people with low A1c before the great east Japan earthquake (A1c < 5.7%) = 85.19, p_1 = 0.8519)

P_2 = Proportions of people with low A1c after the great east Japan earthquake (A1c < 5.7%) = 65.74, p_1 = 0.6574)

Δ = The differenced data between 2 groups (0.8519 - 0.6574 = 0.1945)

$$n = \left(\frac{\{1.96\sqrt{0.8519+0.6574} + 0.842\sqrt{0.8519+0.6574-(0.8519-0.6574)}\}^2}{(0.8519-0.6574)^2} \right)^2$$

n = 311 + missing data 10%

n = 350

This study should recruit at least 350 patients

2.4. Statistical analysis

In this study, the categorical data would be reported in the percentage format. Besides, if it is a normal distribution for the continuous data, the continuous variables would be presented as mean ± standard deviations. For the non-normal distribution, it would be expressed as median (with minimum and maximum).

Chi-square test and Fishers' exact test were performed for the comparison of two groups of categorical data. Student's t-test and Mann-Whitney U- test were performed for comparison of two groups of continuous data. Statistical significance was considered at p-value < 0.05. All statistical analyses were performed by using IBM SPSS Statistics for Windows, version 22.0.

2.5. Definition

2.5.1. Before COVID-19 pandemic

Before the lockdown in Thailand, the government of Thailand announced a nationwide lockdown on March 22, 2020.

2.5.2. During COVID-19 pandemic

The period Between March 22, 2020, and August 22, 2020.

2.5.3. Sustained good control

Patient with A1C is less than 70 g/L (7 %) both before and after the COVID-19 pandemic.

2.5.4. Worse control

Patient with A1C is less than 70 g/L (7 %) before COVID-19 pandemic but greater than or equal to 70 g/L (7 %) after COVID-19 pandemic.

2.5.5. Sustained poor control

Patient with A1C is greater than or equal to 70 g/L (7 %) both before and after the COVID-19 pandemic.

2.5.6. Better control

Patient with A1C is greater than or equal to 70 g/L (7 %) before COVID-19 pandemic but less than 70 g/L (7 %) after COVID-19 pandemic.

3. Results

There were 350 patients included, which were 130 (37.1%) males and 220 (62.9%) females. Baseline characteristics of the patients are presented in Table 1. The mean age ± standard deviation (SD) was 60.8 ± 13.0 years old. Most patients (66%) had oral antidiabetic drug treatment at baseline.

There was significant increase in mean A1C ± SD (74.8 ± 13.7 vs. 76.0 ± 15.3 g/L, p-value <0.016), mean systolic blood pressure (SBP) ± SD (132.26 ± 14.35 vs. 137.64 ±15.65 mmHg, p-value <0.001) and mean diastolic blood pressure (DBP) ± SD (73.41 ± 10.69 vs. 75.60 ± 10.92 mmHg, p-value <0.001) during the COVID-19 virus pandemic. Nevertheless, a statistical difference was not observed in fasting plasma glucose (FPG). The mean duration of outdoor activities ± SD during the COVID-19 virus pandemic was significantly decreased (5.35 ± 4.48 hours per day vs. 4.03 ± 4.37 hours per day, p-value <0.001). Exercise frequencies tend to decrease during the COVID-19 virus pandemic. Parameters compare before and during the COVID-19 pandemic are presented in Table 2.

In our study, subgroup analysis parameters in sustained good control and the worse control group showed nondifferent statistically significant but sustained well control group tends to have more self-monitoring blood glucose (SMBG) and less complication. Most of the patients in this group were covered by the comptroller general's department, and they had more exercised and outdoor activities presented in Table 3.

In subgroup analysis, the parameters compare sustained poor control and better control. The study found statistically significant that the better control group has lower A1C at baseline and fewer outdoor activities during the pandemic than the sustained poor control group. Diabetes control between the two groups shows that sustained poor control group uses oral antidiabetic drugs and Insulin plus oral antidiabetic drugs (53.7% and 33.7%, respectively). However, the better control group uses only oral antidiabetic drugs 79.2% see Table 4

Table 1 Baseline characteristic of patients with T2DM (n = 350

Baseline characteristic	**Value**
Age (years), mean ± SD	60.8 ± 13.0
Male sex, n (%)	130 (37.1%)
Body weight (kilograms), mean ± SD	68.1 ± 15.3
Body mass index (kg/m^2), n (%)	
< 18.5	8 (2.3%)
18.5 - 22.9	73 (20.9%)
23.0 – 24.9	62 (17.7%)
≥ 25	207 (59.1%)
Income (baht), median	15,000 (0-20,0000)
Education, n (%)	
No	20 (6.9%)
Lower bachelor	190 (54.3%)
Bachelor and Upper bachelor	136 (38.9%)
Health insurance, n (%)	
Government	157 (44.9%)
Social security scheme	102 (29.1%)
Universal coverage	55 (15.7%)
State enterprise officer	18 (5.1%)
Self-payment	16 (4.6%)
Non-Thai resident	2 (0.6%)
Comorbidity, n (%)	
No	23 (6.6%)
Yes	327 (93.4%)
Hyperlipidemia	274 (83.8%)
Hypertension	234 (71.6%)
Chronic kidney disease	86 (26.3%)
Coronary artery disease	23 (7.0%)
Cerebrovascular disease	17 (5.2%)

Other	79 (24.2%)
Diabetes control, n (%)	
Oral antidiabetic drugs	231 (66.0%)
Insulin plus oral antidiabetic drugs	75 (21.4%)
Insulin	23 (6.6%)
Diet control	16 (4.6%)
Oral plus GLP1 receptor agonist	5 (1.4%)
Complication, n (%)	
No	197 (56.3%)
Yes	153 (43.7%)
Retinopathy	72 (47.1%)
Nephropathy	75 (49.0%)
Neuropathy	11 (7.2%)
CVD	18 (11.8%)
CAD PAD	26 (17.0%) 4 (2.6%)

Table 2 Comparing parameters before and during COVID-19 pandemic (n=350)

Variables	**Before**	**After**	**Mean difference(95%CI)**	**p-value**
A1c (g/L), mean ± SD	74.8 ± 13.7	76.0 ± 15.3	1.2 (0.2 to 2.1)	0.016*
A1c, n (%)				0.399
A1c < 70 g/L	151 (43.14%)	140 (40%)		
A1c ≥ 70 g/L	199 (56.86%)	210 (60%)		
FPG (mmol/L), mean ± SD	7.95±2.35	8.16 ± 2.65	0.21 (-0.04 to 0.47)	0.097
BMI (kg/m^2), mean ± SD	26.49 ± 5.03	26.52 ± 5.13	0.03 (-0.08 to 0.14)	0.605
BMI (kg/m^2), n (%) < 18.5	8 (2.29%)	10 (2.86%)		0.930
18.5 - 22.9	73 (20.86%)	74 (21.14%)		
23.0 – 24.9	62 (17.71%)	57 (16.29%)		
≥ 25	207 (59.14%)	209 (59.71%)		
SBP (mmHg), mean ± SD	132.26 ± 14.35	137.64 ± 15.65	5.37 (3.91 to 6.83)	< 0.001*
DBP (mmHg), mean ± SD	73.41 ± 10.69	75.60 ± 10.92	2.19 (1.11 to 3.27)	< 0.001*
Medication prescription, n (%)				0.094
Medication delivery	3 (0.86%)	8 (2.29%)		
Pick-up by delegate	15 (4.29%)	25 (7.14%)		
Pick-up by self	331 (94.57%)	314 (89.71%)		
Buy from drugstore	1 (0.29%)	3 (0.86%)		
Outdoor activities (hours/day), mean ± SD	5.35 ± 4.48	4.03 ± 4.37	-1.32 (-1.68 to -0.96)	< 0.001*

Exercise, n (%)				0.210
No	93 (26.57%)	108 (30.86%)		
Yes	257 (73.43%)	242 (69.14%)		
Exercise frequency, n (%)				< 0.001*
Less than once a week	8 (3.1%)	20 (8.3%)		
Once a week	15 (5.8%)	25 (10.3%)		
2-3 times a week	231 (89.9%)	110 (45.5%)		
Everyday	3 (1.2%)	87 (36%)		
Food delivery, n (%)				0.816
No	216 (61.7%)	213 (60.9%)		
Yes	134 (38.3%)	137 (39.1%)		

Table 3 Comparing parameters in sustained well control group and worse control group (n = 151)

	Sustained well control (n = 116)	**Worse control (n = 35)**	**p-value**
Age (years), mean ± SD	63.7 ± 10.6	63.0 ± 12.7	0.746
Sex, n (%)			0.372
Male Female	53 (45.7%) 63 (54.3%)	13 (37.1%) 22 (62.9%)	
Health insurance, n (%)			0.789
Government	61 (52.6%)	15 (42.9%)	
Social security scheme	21 (18.1%)	9 (25.7%)	
Universal coverage	14 (12.1%)	6 (17.1%)	
State enterprise officer	12 (10.3%)	3 (8.6%)	
Self-payment	7 (6.0%)	2 (5.7%)	
Non-Thai resident	1 (0.9%)	0 (0.0%)	
Complication, n (%)			0.477
No	74 (63.8%)	20 (57.1%)	
Yes	42 (36.2%)	15 (42.9%)	
Comorbidity, n (%)			0.685
No	8 (6.9%)	1 (2.9%)	
Yes	108 (93.1%)	34 (97.1%)	
Duration of T2DM (years), mean ± SD	12.4 ± 8.8	10.7 ± 8.5	0.331
Diabetes control, n (%)			0.124
Oral antidiabetic drugs	93 (80.2%)	21 (71.4%)	
Insulin plus oral antidiabetic drugs	11 (9.5%)	3 (8.6%)	
Diet control	10 (8.6%)	3 (8.6%)	
Insulin	2 (1.7%)	3 (8.6%)	
Oral plus GLP1 receptor agonist	0 (0.0%)	1 (2.9%)	
A1c (g/L), mean ± SD	64.0 ± 3.6	65.2 ± 3.6	0.083
FPG (mmol/L), mean ± SD	6.9 ± 1.21	6.85 ± 1.21	0.839
BMI (kg/m^2), mean ± SD	25.65 ± 4.29	24.95 ± 4.60	0.410
SBP (mmHg), mean ± SD	133.26 ± 13.44	134.57 ± 13.97	0.617
DBP (mmHg), mean ± SD	72.82 ± 10.31	70.97 ± 10.57	0.357

Follow up, n (%)			1.000
No	9 (7.8%)	3 (8.6%)	
Yes	107 (92.2%)	32 (91.4%)	
Outdoor activities (hours), mean ± SD	3.19 ± 3.86	2.83 ± 3.78	0.631
Exercise, n (%)			0.179
No	21 (18.1%)	10 (28.6%)	
Yes	95 (81.9%)	25 (71.4%)	
Food delivery, n (%)			0.167
No	75 (64.7%)	27 (77.1%)	
Yes	41 (35.3%)	8 (22.9%)	

Table 4 Comparing parameters in sustained poor control group and better control group (n = 199)

	sustained poor control (n = 175)	**better control (n = 24)**	**p-value**
Age (years), mean ± SD	58.2 ± 14.1	62.7 ± 12.9	0.143
Sex, n (%)			0.738
male	57 (32.6%)	7 (29.2%)	
female	118 (67.4%)	17 (70.8%)	
Health insurance, n (%)			0.284
Government	75 (42.9%)	6 (25.0%)	
Social security scheme	60 (34.3%)	12 (50.0%)	
Universal coverage	31 (17.7%)	4 (16.7%)	
State enterprise officer	2 (1.1%)	1 (4.2%)	
Self-payment	6 (3.4%)	1 (4.2%)	
Non-Thai resident	1 (0.6%)	0 (0.0%)	
Complication, n (%)			0.119
No	87 (49.7%)	16 (66.7%)	
Yes	88 (50.3%)	8 (33.3%)	
Comorbidity, n (%)			0.384
No	11 (6.3%)	3 (12.5%)	
Yes	164 (93.7%)	21 (87.5%)	
Duration of T2DM (years), mean ± SD	11.9 ± 8.6	10.2 ± 7.9	0.353
Diabetes control, n (%)			0.047*
Oral antidiabetic drugs	94 (53.7%)	19 (79.2%)	
Insulin plus oral antidiabetic drugs	59 (33.7%)	2 (8.3%)	
Diet control	3 (1.7%)	0 (0.0%)	
Insulin	16 (9.1%)	2 (8.3%)	
Oral plus GLP1 receptor agonist	3 (1.7%)	1 (4.2%)	
A1c (g/L), mean ± SD	84.1 ± 13.3	73.7 ± 3.8	< 0.001*
FPG (mmol/L), mean ± SD	8.86 ± 2.77	7.98 ± 1.45	0.839
BMI (kg/m^2), mean ± SD	27.17 ± 5.48	27.81± 4.48	0.582

SBP (mmHg), mean ± SD	130.90 ± 15.05	134.04 ± 13.68	0.333
DBP (mmHg), mean ± SD	74.15 ± 11.11	74.19 ± 9.40	0.912
Follow up, n (%)			0.540
No	27 (15.4%)	2 (8.3%)	
Yes	148 (84.6%)	22 (91.7%)	
Outdoor activities (hours/day), mean ± SD	4.28 ± 4.48	2.17 ± 2.94	0.026*
Exercise, n (%)			0.487
No	56 (32%)	6 (25%)	
Yes	119 (68%)	18 (75%)	
Food delivery, n (%)			0.322
No	98 (56%)	16 (66.7%)	
Yes	77 (44%)	8 (33.3%)	

4. Discussion

The study's main finding was a significant increase in mean A1C, mean SBP, and mean DBP during the COVID-19 virus pandemic while the duration of outdoor activities was significantly decreased and exercise frequencies decreased during the COVID-19 virus pandemic. The impact of quarantine, social distancing, and community containment during the epidemic on lifestyles may be the essential factors in the increase of A1C.

Our results are similar to a retrospective cohort study of people with T2DM who attended one of five tertiary hospitals in Daegu during the COVID-19 virus pandemic. The study found the change in A1C in people with T2DM who undertook social distancing because of COVID-19. The A1c during the COVID-19 virus pandemic was significantly higher [11] · However, in a center, retrospective, observational study in Turkey for Type 2 DM patients aged between 18-80 years in terms of glycemic parameters, A1c rose from 76.7 ± 17.6 to 81.1 ± 24.8 g/L, and fasting glucose from 8.76 (4.61-35.8) mmol/L to 9.06 (4.66-30.53) mmol/L, none of which were statistically significant ($p=0.253$, $p = 0.079$, respectively). From none statistically significant rose of A1C and FPG in the study in Turkey might relate to the number of population in the study, 101 patients were in the study [12] . Another study in China by Ting Xue et al., 2020 studied T2DM patient's ages more than 65 years during the COVID-19 pandemic. The study collected data from Fujian hospital; fasting plasma glucose and A1c were collected from the patient before the pandemic January 1, 2019, to March 8, 2019, compared with the same duration in 2020. There were 135 patients in this study. The results showed higher FPG statistically significant during the COVID-19 pandemic than pre-pandemic. Also, A1c was higher but not statistically significant (72.0 ± 17 to 74 ± 18 g/L, p-value 0.158) [9]. In Italy, a retrospective study in T1DM that included 13 individuals with a median age of 14.2 years showed metabolic control of T1DM in adolescents using the hybrid closed loop system did not worsen during the restrictions due to the COVID-19 pandemic and further improved in those who continued physical activity during the quarantine [13].

In addition to the study of viral pandemics affecting the blood sugar level, the study of Medicine faculty, Thammasat University, monitored the blood sugar level during the flood disaster in 2011. There were 300 T2DM patients. The result showed that 19% discontinued their medicine. In good compliance, the group reported the rising of FPG during the flood disaster (8.49 mmol/L) compared with pre-disaster (7.88 mmol/L) significantly (<0.001). On the other hand, bodyweight and HDL-C were better than in the pre-disaster period. In discontinue of medicine, the group reported FPG and A1C were statistically significantly higher during flood disaster [14]. In other studies, the effect of flood disaster on blood sugar control in England 2007, including 1743 patients, reported A1C was lower in pre-disaster period statistically significant. [76 (75–77) g/L vs. 79 (77–80) g/L, $p = 0.002$] [15].

The previous research found that the disaster or pandemic disease affects blood sugar control or A1C. Our research reported that the COVID-19 pandemic increased A1C and decreased outdoor activity. This study related to a short-term observational cohort study at the Leiden University Medical Center, which found less exercise in both people with relatively well-controlled type 1 and type 2 diabetes during short-term lockdown measures [16]. Due to less activity cause a sedentary lifestyle, even patients with good medication compliance engender poor blood sugar and blood pressure control statistically significant.

In our study, subgroup analysis parameters in sustained good control and the worse control group showed nondifferent statistically significant but in sustained good control group tend to have more SMBG and minor complications. Most of the patients in this group were covered by the comptroller general's department and had more exercise and outdoor activities. This study also indicated that patients with blood glucose monitoring devices tend to have more minor complications and optimal A1c, although the COVID-19 pandemic. Compared with sustained poor control and better control, the latter have lower A1c at baseline and had less outdoor activity during the COVID-19 pandemic. Better control uses oral antidiabetic drugs at baseline more than sustained poor control. However, there were no collected data on subgroup analysis about medication adjustment. Because in the better control group, the better A1C may be related to the medication adjustment.

The strength of this study is data collection. The study collected all 350 patients' A1C, FPG, and BP before and during the COVID-19 pandemic. Since FPG may vary by food or body activity in short period, but A1C reflects average glycemia over approximately three months. The test is the primary tool for assessing glycemic control and has a substantial predictive value for diabetes complications [17]. So, we focused on the primary outcome as A1C, decided the exclusion criteria to exclude some disease or condition that may interfere with A1C, and we collected A1C before the pandemic and during the pandemic every two months.

The limitation of this study is the study design. The small observational study has some confounding factors. Patient data were collected from self-pick-up medicine at the hospital. Most of them usually took good care of themselves, which may not represent all diabetic patients missed data related to medication adjustment and lack of metabolic profile data, for example, lipid profile also, no record data about patient diet control and some specific activity. A researcher would recommend collecting more data about lipid profile, using a standard questionnaire, focusing more on diet information, exercise, stress, and other factors that may affect blood pressure.

This study's advantage reminds us to realize the importance of glycemic control during the COVID-19 pandemic. Although this study showed significant differences before and during the COVID-19 pandemic, the patient cannot do regular outdoor activity. Lockdown social distancing and community containment might change to a sedentary lifestyle. Healthcare providers should recommend patients to do home exercise, self-monitoring blood glucose, and blood pressure regularly. The study indicated the higher A1C and BP during the COVID-19 pandemic statistically significant.

5. Conclusion

The present study showed that mean A1C was significantly increased during the COVID-19 virus pandemic. Nevertheless, a statistical difference was not observed in FPG. The impact of quarantine, social distancing, and community containment during the epidemic on lifestyles may be the most crucial factor in increasing A1C. Both doctors and patients should pay more attention to the management of T2DM during the COVID-19 virus pandemic.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that there is no conflict of interest.

Statement of ethical approval

This study was approved by Rajavithi Hospital ethic committee number 114/2563.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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