Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren[1–3]

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ABSTRACT

Background: To our knowledge, no rigorously designed clinical trials have evaluated the relation between vitamin D and physician-diagnosed seasonal influenza.

Objective: We investigated the effect of vitamin D supplements on the incidence of seasonal influenza A in schoolchildren.

Design: From December 2008 through March 2009, we conducted a randomized, double-blind, placebo-controlled trial comparing vitamin D_3 supplements (1200 IU/d) with placebo in schoolchildren. The primary outcome was the incidence of influenza A, diagnosed with influenza antigen testing with a nasopharyngeal swab specimen.

Results: Influenza A occurred in 18 of 167 (10.8%) children in the vitamin D_3 group compared with 31 of 167 (18.6%) children in the placebo group [relative risk (RR), 0.58; 95% CI: 0.34, 0.99; P = 0.04]. The reduction in influenza A was more prominent in children who had not been taking other vitamin D supplements (RR: 0.36; 95% CI: 0.17, 0.79; P = 0.006) and who started nursery school after age 3 y (RR: 0.36; 95% CI: 0.17, 0.78; P = 0.005). In children with a previous diagnosis of asthma, asthma attacks as a secondary outcome occurred in 2 children receiving vitamin D_3 compared with 12 children receiving placebo (RR: 0.17; 95% CI: 0.04, 0.73; P = 0.006).

Conclusion: This study suggests that vitamin D_3 supplementation during the winter may reduce the incidence of influenza A, especially in specific subgroups of schoolchildren. This trial was registered at https://center.umin.ac.jp as UMIN000001373. *Am J Clin Nutr* 2010;91:1255–60.

INTRODUCTION

Seasonal oscillation of influenza is prominent, its epidemic is explosive, and it ends abruptly. To explain this peculiar pattern, Cannell et al (1) hypothesized that the seasonal oscillation of serum vitamin D concentrations, which was recently discovered to up-regulate innate immunity, may affect the epidemic pattern of influenza. Vitamin D is mostly obtained from sun exposure; thus, serum vitamin D concentrations can be affected by season. In fact, serum concentrations of vitamin D have been shown to decrease in winter, the season when influenza occurs, to concentrations half those during the summer (1). In a post hoc analysis of side effect questions asked during a randomized controlled trial performed to determine whether vitamin D could prevent osteoporosis (2), cold and flu symptoms were reported 3 times less often in the vitamin D group than in the placebo group (3). However, although the authors conducted an additional randomized trial in 162 healthy adults, they could not reconfirm the benefit of vitamin D supplementation for the prevention of symptomatic upper respiratory tract infections (4). On the other hand, a significant inverse association between serum vitamin D intake and recent upper respiratory tract infections was seen in the third National Health and Nutrition Examination Survey (5). However, no rigorously designed clinical trials have evaluated the relation between vitamin D and physician-diagnosed influenza or delineated the necessary changes to prepare for an influenza pandemic (6). We conducted a randomized, double-blind, placebo-controlled trial comparing vitamin D_3 supplements with placebo in schoolchildren to elucidate whether preventive intake of vitamin D supplements during winter and early spring seasons can reduce the incidence of seasonal influenza A.

SUBJECTS AND METHODS

Study design

A multicenter, randomized, double-blind, placebo-controlled, parallel-group trial was conducted by 12 hospitals and 8 doctors in private practice in Japan over 4 mo (from 1 December 2008 to 31 March 2009). The study protocol was reviewed and approved by the ethics committee of all participating hospitals. The entire process of study design and protocol, data monitoring, and analyses was performed only by academic authors; there was no industry support or involvement in the study. The data monitoring center was at the Division of Molecular Epidemiology, Jikei University School of Medicine. The safety review board consisted of 2 physicians from the Jikei University Hospital, who are not coauthors of this study. Both vitamin D_3 (Status D_3) and placebo were purchased by the academic study group from Zenyaku Co, Ltd (Otsuka, Bunkyo-ku, Tokyo, Japan).

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Study population, eligibility, and consent

Schoolchildren aged 6–15 y, with or without underlying diseases, were eligible and asked to participate in the study by the pediatricians in charge of the outpatient clinics. The accrual period was from 1 November 2008 to 15 December 2008. Participants were asked to start taking the study drugs between 1 and 15 December 2008 and to continue taking the medicine until 31 March 2009. Children were excluded if they *1*) had a history of stones in the urinary tract or diseases of calcium or bone metabolism, *2*) were already taking vitamin D_3 or activated vitamin D as a treatment of an underlying disease, *3*) had a history of allergic reactions to ingredients in the tablets, *4*) had difficulties swallowing tablets, *5*) had been receiving immunosuppressive therapy including oral corticosteroids or chemotherapy within the past year, and *6*) were considered incapable of taking part in the study by the pediatrician in charge. Parents and children were asked to provide written informed consent after the pediatrician explained the study to them at the outpatient clinic.

Randomization, blinding, and intervention

We used a central computerized procedure to randomly assign children in permutated blocks of 4 to receive either vitamin D_3 or placebo. Parents were provided with 8 numbered bottles, each of which contained 90 tablets. One tablet contained 200 IU vitamin D_3 or placebo, and the active and placebo tablets were identical in appearance. The participants were asked to take 3 tablets twice daily (total: 1200 IU vitamin D_3 or placebo). Blinding of the study was achieved by bottle labeling. The randomization code was disclosed to the staff at the data monitoring center after labeling the number on each bottle. Staff at the data monitoring center had no contact with the patients.

Follow-up procedures and ascertainment of outcomes

The participants' parents were asked to send back the prestudy questionnaires just after randomization for an assessment of *1*) basic data, such as sex, age, weight, and height; *2*) family structure; *3*) medical history, including atopic dermatitis, otitis media, sinusitis, asthma from age 3 y and older, and other underlying diseases; and *4*) skin reaction to sun exposure (ie, level of sunburn). To help increase compliance, participants and their parents were asked to show the bottles of supplements when they visited the medical facilities, where they were given the study supplements. One bottle was to be consumed in 15 d. They were also asked to return the poststudy questionnaires after March 31 for an assessment of *1*) diagnosis by pediatricians of primary and secondary outcomes; *2*) adherence with study drug; *3*) frequency of outdoor activities per week; *4*) average frequency of intake of specific dietary items per week, including sun-dried or fresh shiitake mushrooms, salmon, sardines, mackerel, tuna, and egg yolk; and *5*) days absent from school. A log was completed daily that included the following information: adherence to study drug, days absent from school, times of visits to clinics or hospitals, hospital admissions, and cases of influenza, fever, asthma attack, and gastroenteritis (nausea, vomiting, and diarrhea). For case identification, the study number was used and private information such as the names and addresses of the participants was not disclosed to the data monitoring center.

Basic information (age, sex, height, weight, and underlying disease if present) concerning the participants was sent to the data monitoring center by the pediatrician in charge at entry by using the identification number for this study. The primary outcome was influenza A, diagnosed by medical doctors using a rapid influenza diagnostic test (RIDT) with a nasopharyngeal swab specimen, on an outpatient basis, following the manufacturer's protocol. Collaborating medical institutions were asked to use a kit with both sensitivity and specificity >95%. Influenza B diagnosed via nasopharyngeal swab was included as a secondary outcome. We defined RIDT-negative influenza-like illness as RIDT-negative cases suspected by doctors due to clinical signs such as fever, headache, arthralgia, runny nose, and/or coughing as well as close contact with patients with influenza. Other secondary outcomes were physician-diagnosed *1*) asthma attack that included wheezing improved by inhalation of a β stimulant in patients who already had a diagnosis of asthma, *2*) nonspecific febrile ($\geq$38.5°C at least once) infection in those who were not suspected to have influenza as well as other specific diseases and thus did not undergo RIDT, *3*) gastroenteritis with $\geq$2 of 3 symptoms (nausea or vomiting, diarrhea, or fever $\geq$37.0°C), *4*) pneumonia diagnosed with chest X-ray, and *5*) admission to the hospital for any reason. When pediatricians made a diagnosis of one of these outcomes, they sent a fax to the data monitoring center. In case of adverse events, including urinary tract stones and other serious signs/symptoms, pediatricians were asked to send a fax to the data monitoring center. When the occurrence of the primary outcome was described only in returned poststudy questionnaires but not sent via fax by the pediatricians, the data monitoring center reconfirmed the outcome by direct communication with the pediatrician in charge by using the study identification number in a blinded fashion to the randomization code. The participants and their parents were asked to visit the medical facilities whenever the participants had a fever.

Statistical analysis

We estimated that the primary outcome would occur in 20% of children in the placebo group. An equally divided sample of 480 was calculated as being sufficient for the detection of a 50% reduction in outcome, with a type I error (2-sided) of 5% and a power of 80%, on the assumption of a 10% loss to follow-up. Interim analyses were not used because the study period lasted only 4 mo.

Efficacy was assessed by using an intention-to-treat analysis. Continuous variables were compared by using Wilcoxon's rank-sum test, and categorical variables were assessed with the chi-square test. The incidences of both primary and secondary outcomes were compared in the 2 groups by using relative risks (RRs) and 95% CIs, subgrouped by sex, age, and nonasthma or asthma. We tested the null hypothesis of equality of risk ratios between the demographic groups compared by using a chi-square test. All reported P values were 2-sided. P values <0.05 were considered statistically significant. No adjustments were made for multiple comparisons. All analyses were performed by using Stata 9.0 (StataCorp LP, College Station, TX).

RESULTS

Characteristics of the study population

A total of 430 schoolchildren/parent pairs who met the inclusion criteria agreed to participate in this study and were

randomly assigned to treatment (**Figure 1**). Age, sex, weight, height, number in the family, number of siblings, starting age of nursery school, skin reaction to sun exposure, and underlying diseases including medical history from age 3 y of atopic dermatitis, otitis media, sinusitis, and asthma, based on questionnaires, were similar between the 2 groups (**Table 1**). The mean age of the study population was 10.2 y, 56% were male, the mean family size was 4.5 persons, 23% had no siblings, and 65% started nursery school or kindergarten at 3 y of age or older. A total of 27% had underlying diseases and 26% had bronchial asthma, all of which were diagnosed by pediatricians. A total of 56% and 23% of participants had a history of asthma and atopic dermatitis, respectively, after the age of 3 y. There were no significant differences in baseline characteristics between the 2 groups as assessed by chi-square tests and Wilcoxon's rank-sum test.

Adherence

Of the 430 children, 334 were followed until the end of the study. Loss to follow-up occurred for 50 children in the vitamin D_3 group and 46 in the placebo group ($P = 0.72$). Compliance with taking vitamin D or placebo twice daily was evaluated on the basis of the logs provided, which showed that 96% took the drug as directed. Compliance patterns did not differ significantly between the 2 groups ($P = 0.23$). Children who were already taking vitamin D_3 or activated vitamin D as a treatment of underlying diseases were excluded before randomization. However, some participants started taking vitamin D supplements in addition to the study supplement after randomization, because this was not prohibited.

Primary outcome

Influenza A occurred in 49 children. The first case occurred on 15 December 2008. The incidence of influenza A peaked from the middle to late weeks of January. Influenza A occurred in 18 of 167 (10.8%) children receiving vitamin D_3 compared with 31 of 167 (18.6%) children receiving placebo (RR: 0.58; 95% CI; 0.34, 0.99; $P = 0.04$) (**Table 2**). We compared the incidence of influenza A based on the timing of onset of disease symptoms relative to the initiation of vitamin D intake after supplementation started. Between day 1 and day 30, the occurrence of influenza A was not significantly different between the vitamin D_3 group (2/167; 1.2%) and the placebo group (4/167; 2.4%). Between day 31 and day 60, influenza A occurred significantly less often in the vitamin D_3 group (9/167; 5.4%) than in the placebo group (22/167; 13.2%) (RR: 0.41; 95% CI: 0.19, 0.86; $P = 0.014$). Between day 61 and the end of the study, the occurrence of influenza A was not significantly different between the vitamin D_3 group (7/167; 4.2%) and the placebo group (5/167; 3.0%).

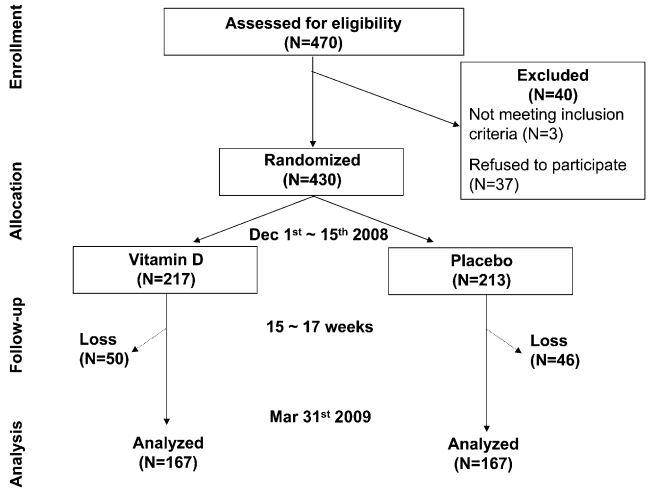

FIGURE 1. Participant flow.

The study population was subgrouped by factors. Significant results are shown in Table 2. The effect of reducing the incidence of influenza A was more prominent in children who had not been taking additional vitamin D supplements other than the study drug (RR: 0.36; 95% CI: 0.17, 0.79; $P = 0.006$) and who started nursery school at age 3 y or older (RR: 0.36; 95% CI: 0.17, 0.78; $P = 0.005$). On the other hand, other subpopulations—male compared with female, <10 compared with ≥ 10 y of age, no older siblings compared with at least one older sibling, university-affiliated hospitals compared with private practices and urban areas (Tokyo, Chiba, Kanagawa, Saitama Prefectures) compared with rural areas (Shizuoka, Niigata, Hokkaido)—showed no significant effect modifications.

Secondary outcomes

Secondary outcomes for both groups are shown in **Table 3**. The incidences of influenza B and RIDT-negative influenza-like illness were not significantly different between the vitamin D_3 and placebo groups. Asthma attacks occurred in 2 children receiving vitamin D_3 compared with 12 children receiving placebo (RR: 0.17; 95% CI: 0.04, 0.73; $P = 0.006$) in children with a previous diagnosis of asthma. There were no significant differences in any other secondary outcomes between groups. There were no reports of adverse events in our study.

DISCUSSION

In this randomized clinical trial, daily supplementation with 1200 IU vitamin D_3 in school children between December and March showed a significant preventive effect against influenza A, although no significant difference was observed for influenza B. A 10-d course of postexposure prophylaxis with zanamivir or oseltamivir resulted in only an 8% decrease in the incidence of symptomatic influenza in children (7). In contrast, daily dietary probiotic supplementation was a safe effective way to reduce fever and other symptoms in small children (8). Moreover, a significant preventive effect of a product containing echinacea, propolis, and vitamin C on the incidence of respiratory tract infections was observed in children (9).

Several plausible mechanisms might explain how vitamin D could have reduced the risk of influenza A. Vitamin D increases the production of antimicrobial peptides such as defensin in primary human monocytic and epithelial cells (10). Moreover, defensin inhibits influenza virus infections by blocking membrane fusion mediated by viral hemagglutinin (11–13). Therefore, in this study, vitamin D supplementation possibly enhanced innate immunity by up-regulating antimicrobial peptides, including defensin, and protected children from influenza A infection. Moreover, vitamin D was reported to reduce inflammation by regulating cytokine release (14–17). Thus, vitamin D may soften the clinical symptoms

TABLE 1
Characteristics of the study population

	Vitamin D_3[1] (n = 217)	Placebo[1] (n = 213)	P value
Age (y)	10.0 ± 2.2[2,3]	10.4 ± 2.4	0.07[3]
Male [n (%)]	124 (57)	118 (55)	0.72[4]
Weight (kg)	35.2 ± 18.3	35.9 ± 13.0	0.36[3]
Height (cm)	137.7 ± 13.8	139.7 ± 18.1	0.14[3]
No. in family	4.6 ± 1.2	4.5 ± 1.5	0.85[3]
Siblings [n (%)]	45 (21)	55 (26)	0.21[4]
No. of siblings			
Older: 0/1/2/3	127/66/23/1	134/63/15/1	0.59[4]
Younger: 0/1/2/3/4	109/75/29/4/0	108/75/25/4/1	0.87[4]
Starting age of nursery school [n (%)]			0.21[4]
<3 y	6 (35)	61 (29)	
≥3 y	138 (64)	144 (68)	
Underlying diseases [n (%)]	58 (27)	58 (28)	0.82[4]
Bronchial asthma	51 (24)	59 (28)	0.32[4]
Other	7 (3)[5]	9 (4)[6]	0.51[4]
Medical history based on questionnaires [n (%)][7]			
Atopic dermatitis	46 (21)	52 (24)	0.30[4]
Otitis media	44 (20)	40 (19)	0.73[4]
Sinusitis	46 (21)	31 (15)	0.11[4]
Asthma	117 (54)	113 (53)	0.87[4]
Skin reaction to sun exposure [n (%)]			
Sunburn but no suntan	6 (3)	12 (6)	0.30[4]
Sunburn, slight suntan, recover in winter	52 (25)	55 (28)	
Sunburn, average level of suntan	128 (62)	109 (56)	
No sunburn, always suntan	20 (10)	20 (10)	

[1] The additive materials consisted of gelatin, D-sorbitol, yellow-5, sesame oil, and titanium dioxide. In addition, 1200 IU vitamin D_3 was included in the active tablets.
[2] Mean ± SD (all such values).
[3] Data were evaluated with Wilcoxon's rank-sum test.
[4] Data were evaluated with the chi-square test.
[5] Type I diabetes, dwarfism (n = 2), hemophilia A, mental retardation + epilepsy, sequelae of encephalitis, and allergic purpura.
[6] Attention-deficit hyperactivity disorder, mental retardation, sequelae of encephalitis, congenital heart disease, epilepsy, Hirschsprung disease, anorexia nervosa, pituitary dwarfism, and nocturnal enuresis.
[7] Previous history diagnosed from age 3 y.

and signs of influenza by reducing cytokine secretion. In this study, vitamin D reduced the incidence of influenza A, but not of influenza B. The cytokine secretion pattern can differ between influenza A and influenza B (18). If vitamin D modulates cytokine secretion, preventive effects of vitamin D may be different between influenza A and influenza B.

Because taking vitamin D_3 supplements for 1 y with a dose ranging from 200 to 2000 IU in schoolchildren has been shown to be safe (19), we set the dose of vitamin D_3 at 1200 IU in this trial. No serious adverse events occurred. It reportedly takes ≈3 mo to reach a steady state of vitamin D concentrations by supplementation (20). Thus, December might be theoretically

TABLE 2
Influenza A as a primary outcome, subgrouped by factors

	Subjects with influenza A					
	Vitamin D_3	Placebo	Relative risk	95% CI	P value	P value[1]
	n/total n (%)					
Influenza A	18/167 (10.8)	31/167 (18.6)	0.58	0.34, 0.99	0.04	
Additional vitamin D[2]						0.04
None	8/140 (6.0)	22/140 (16.5)	0.36	0.17, 0.79	0.006	
At least once per week[3]	10/34 (29.4)	9/34 (26.5)	1.11	0.52, 2.39	0.79	
Starting age of nursery school						0.04
<3 y	10/59 (16.9)	7/49 (14.3)	1.19	0.49, 2.88	0.71	
≥3 y	8/107 (7.5)	24/117 (20.5)	0.36	0.17, 0.78	0.005	

[1] We compared the null hypothesis of equality of risk ratios between demographic groups by using a chi-square test.
[2] Vitamin D supplement other than the study drug.
[3] Frequencies: 200–400 IU/wk (n = 7), 600–800 IU/wk (n = 7), 1000–1200 IU/wk (n = 46), and ≈1400 IU/wk (n = 8).

TABLE 3
Secondary outcomes[1]

	Outcome				
	Vitamin D_3	Placebo	Relative risk	95% CI	*P* value
	n/total n (%)				
Influenza B	39/167 (23.3)	28/167 (16.8)	1.39	0.90, 2.15	0.13
RIDT-negative influenza-like illness	8/167 (4.8)	9/167 (5.4)	0.89	0.35, 2.25	0.80
Nonspecific febrile disease	4/167 (2.4)	5/167 (3.0)	0.80	0.22, 2.93	0.74
Asthma attack	2/167 (1.2)	12/167 (7.2)	0.17	0.04, 0.73	0.006
Gastroenteritis	11/167 (6.6)	15/166 (9.0)	0.73	0.35, 1.54	0.41
Pneumonia	2/167 (1.2)	2/167 (1.2)	1.00	0.14, 7.01	1.00
Admission to hospital	1/167 (0.5)	3/167 (1.8)	0.33	0.04, 3.17	0.31
Days absent from school (*n*)					0.39[2]
0	86	96			
1–5	66	54			
≥6	15	17			

[1] RIDT, rapid influenza diagnostic test.
[2] Calculated by chi-square test.

too late to start supplementation. However, in this study, vitamin D_3 significantly reduced the incidence of influenza A within 60 d.

The preventive effect of vitamin D was consistent and more prominent in some subgroups of children, including those who had not been taking vitamin D supplements other than the study drug, thus making it reasonable to consider the effect of cointervention. Similarly, children who started nursery school before age 3 y might have had a higher chance of exposure to influenza and of obtaining the immunity than children who started after 3 y.

In children who did not have asthma, the incidence of influenza A was reduced by vitamin D_3 (data not shown). However, the incidence was not significantly reduced in children with asthma. Children with asthma may be more susceptible to influenza (21). After experimental infection with influenza A virus, interleukin-10 production was shown to be significantly lower in subjects with allergies than in subjects without allergies (22). Many genes involved in the vitamin D pathway, such as interleukin-10, seem to be common with asthma and atopy (23).

In contrast, asthma attacks were significantly suppressed by vitamin D_3. Asthma attacks occurred in patients with asthma who were diagnosed by doctors before starting the study: there were no cases of new-onset asthma. Black and Scragg (24) showed that as serum vitamin D concentrations increase, forced expiratory volume in 1 s also increases. Camargo et al (25) showed that children of women who had vitamin D deficiency during pregnancy and were living in an inner city were at an increased risk of wheezing illnesses. Vitamin D insufficiency was relatively frequent in an equatorial population of children with asthma, in whom lower vitamin D concentrations are associated with elevated markers of allergy and asthma severity (26).These previous results are not inconsistent with our findings. Activated vitamin D was shown to decrease airway smooth muscle cell growth in vitro (27). No intervention trials to prevent asthma attacks using vitamin D supplements have been conducted (28), and our preliminary data may further support putting a full scale of randomized controlled trial into practice.

The major limitations of the present study were a *1*) small sample size; *2*) lack of serum 25-hydroxyvitamin D data; *3*) lack of urinary calcium data; and *4*) lack of information on the presence or development of influenza A antibodies. First, our original population was not large. In addition, when we subdivided the study population into 2 groups, the sample size and event number were so small that the chance of random effects unrelated to the intervention could have increased. In addition, follow-up rates were <80%, although the rates were similar in both groups and higher than we anticipated. For schoolchildren, it might be hard to continue taking medicine for preventive reasons for >3 mo. Moreover, the comorbidity ratio of the study population was relatively high. It is possible that because university and general hospitals collaborated in this study, most participants were enrolled at outpatients. The evidence obtained in this study thus may not be generalizable to other populations. Second, serum concentrations of 25-hydroxyvitamin D_3 were not measured; thus, we do not know the exact threshold of serum concentrations needed to decrease the incidence of influenza A. In addition, compliance could only be assessed by using the daily logs provided by the children and at times of presentation of illness, which could have caused bias toward the null hypothesis. Third, although no adverse events (including urinary stones) were reported, urinary calcium concentrations were not measured; thus, we had no data on hypercalciuria as an adverse event. Finally, we did not measure serum antibody concentrations to influenza at the beginning or the end of the study. Thus, we do not know how many mild and asymptomatic forms were included in the study population.

Future studies should include a larger sample size of schoolchildren without comorbidities to determine the optimal dose and duration of vitamin D supplementation by measurement of serum 25-hydroxyvitamin D, serum and urinary calcium, and titers of antibody to influenza levels.

In conclusion, our study suggests that vitamin D_3 supplementation during the winter season may reduce the incidence of influenza A. This effect was prominent in specific subgroups of schoolchildren. Moreover, asthma attacks were also prevented by vitamin D_3 supplementation.

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The authors' responsibilities were as follows—MU: study design, data analysis and interpretation, writing of the manuscript, and final approval of the manuscript; and TS, MO, MK, YW, and HI: recruitment of participants, data collection, review of the original data and their compilation, and final approval of the manuscript. None of the authors had any conflicts of interest.

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