Original Article

Micronutrients decrease incidence of common infections in type 2 diabetes outpatients

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A randomized, double-blind, placebo-controlled trial was carried out to investigate the effects of micronutrients supplementation on immunity and the incidence of common infections in type 2 diabetic outpatients. A total of 196 type 2 diabetic outpatients were randomized to receive tablets of micronutrients (n=97) or placebo (n=99) for 6 months. Individualized dietary energy intake and daily physical activity were recommended. Anthropometric measurements, blood biochemical variables and the incidence of common infections were measured at baseline and at 6 months. Data on diet, exercise and infection (upper respiratory tract infection, skin infection, urinary and genital tract infections, other infections) were recorded 1 month before the study and every month during the study. Blood concentrations of total protein, iron (Fe), folic acid and hemoglobin increased and unsaturated iron-binding capacity(UIBC) levels were decreased in the micronutrients supplementation group compared to the placebo group at 6 months. Moreover, at 6 months, compared to the placebo group, the blood concentrations of IgE, CD^{4+}, CD^{4+}/CD^{8+}, WBC, lymphocyte counts, basophilic leukocyte increased and CD^{8+} count decreased in the supplementation group, and the levels of IgA, IgM, IgG and complements C3 and C4 did not differ. The incidence of upper respiratory infection, whitlow, dermapostasis, vaginitis, urinary tract infection, gingivitis and dental ulcer were lower and body temperature and duration of fever greatly improved in the supplementation than the placebo group. These data indicated that supplementation of micronutrients might increase immune function and reduce the incidence of common infections in type 2 diabetic outpatients.

Key Words: type 2 diabetes mellitus, micronutrients, infection, immune function, nutrition

INTRODUCTION

Type 2 diabetes mellitus (DM) predisposes patients to increased susceptibility to various infections.[1,2] Some of infections are more likely to have a complicated course in DM than in non-DM patients.[3,4] For example, diabetic ketoacidosis is precipitated or complicated by infection in 75% of cases,[3] and mortality of patients with infection and ketoacidosis is 43%.[3] The pathological mechanisms responsible for such high incidence and severe infections in diabetic patients are still unknown. The best explanation for these are defects in immunity, increased adherence of microorganisms in DM patients, presence of micro- and macro-angiopathy or neuropathy, and a high number of medical interventions in DM patients.[4]

Nutrition may play a critical role in maintaining normal immune function. Many micronutrients such as vitamins A, D and E, β-carotene, folic acid, zinc (Zn), selenium (Se), iron (Fe) and copper (Cu) are important for several components of innate immunity.[5-8] Supplementation with a variety of vitamins or multivitamin-mineral preparations may lower the incidence of infection or reverse some of the changes associated with impaired immune responses in elderly people.[5,8-10] Barringer reported that a multivitamin-mineral supplement reduced the incidence of infection and improved quality of life in 51 patients with type 2 DM.[9] This finding might have considerable practical significance for DM patients because of the lower cost and relatively safe nature of multivitamin-mineral supplements.

We performed a large clinical trial to confirm whether supplementation with micronutrients could improve immune function and decrease the incidence of common infections in outpatients with type 2 DM.

MATERIALS AND METHODS

Subjects and Protocol

We consecutively enrolled 196 patients with a diagnosis of type 2 DM who were outpatients at the Chinese People's Liberation Army (PLA) General Hospital. Other enrollment criteria included (1) age ≥45 years; (2) body mass index ≥22 kg/m^2; (3) both male and female; (4) no history of hepatic, renal or gastrointestinal diseases or diabetic ketoacidosis or no diabetic foot ulcers; no severity

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of chronic complications of DM during the study; (5) no oral administration of multivitamins or minerals for more than 2 to 3 weeks in the month before the enrollment.

All subjects recruited were willing to be randomly assigned to receive micronutrient or placebo tablets, and were required to sign their written consent before starting the study. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethnics Committee of Chinese PLA General Hospital. Before the study, diet, medication, cigarette smoking and alcohol drinking of all patients enrolled in our study were investigated. This data were defined as baseline data.

The study followed a randomized, placebo-controlled, double-blind design. Subjects were randomly allocated to receive micronutrient or placebo tablets by random numbers assigned by the providers of the micronutrient and placebo tablets, who also encoded the tablets with matching random numbers. Neither the subjects nor the researchers of this study knew which subject was receiving which tablet during the study. The micronutrient and placebo tablets were provided by Wuxi Jiante pharmaceutical Co Ltd, and the micronutrient tablets which contained: vitamin D, 2.5 μg; vitamin E, 6.5 mg; vitamin B-1, 0.5 mg; vitamin B-2, 0.5 mg; vitamin B-6, 0.5 mg; vitamin C, 50 mg; folic acid, 75 μg; calcium, 200 mg; iron, 3 mg; Zn, 3 mg; and Se, 12.5 μg, were approved to be a health food supplement by the Chinese Food and Drug Administration (0276, 2001). Patients were asked to take the tablets orally twice each day, in the morning and afternoon, for 6 months.

All patients were informed of the study procedures, provided a manual of protocol, and asked to keep medications for diabetes treatment. The patients were requested to maintain a relatively balance diet and fixed daily physical activity during the 6 months of the study. The patients were instructed to record the type and time of daily physical activity and the content and weight or volume of daily meals in dietary diaries for 3 days (including one weekend day) in one month before the study and every month during the study. The diaries were collected to confirm whether patients followed the instructions, and if necessary, patients were asked again to maintain their diet and physical activity. The patients who could not follow the instructions of diet and physical activity or taking tablets for more than 7 days were asked to drop out of the study. Daily intake of energy, protein, fat, carbohydrates and micronutrients was calculated on the basis of the Tables of Chinese Food Composition, published in 2002 and 2004.

Collection of data on infections

A detailed pamphlet was distributed to all patients to instruct them on how to record infections and symptoms at the beginning of the study, and patients were advised to call the researcher if they had any questions or were uncertain. Infections and symptoms or signs were recorded by patients on the specific forms attached to the pamphlet. The forms were reviewed by investigators for completeness on the day of the study visit, each month. Using these forms, the investigators made a specific diagnosis of infection. If a diagnosis of infection was made, the patients were required to visit their own physicians to receive treatment for the infections. Standard criteria were used to diagnose common infections (upper respiratory tract infection, skin infection, urinary and genital tract infections, other infections). Symptom records that fit into the above categories were confirmed by the study physician to exclude other infectious and noninfectious diseases (eg, allergic rhinitis and diarrhea induced by medications). Symptoms and signs such as fever, body temperature during fever, days of fever and sore throat were also recorded. The incidence of infection was defined as the average percentage of the total number of infections diagnosed by the physician at baseline and every month during the study for both groups. The body temperature during fever and days of fever were the average of absolute figures.

Anthropometric measurements

Anthropometric measurements of body weight, height, waist circumference (WC), hip circumference (HC), and body mass index (BMI) were measured or calculated at baseline and at 6 months. Overnight-fasted subjects were asked to wear underwear for measurement in the morning. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Waist circumference was measured at the umbilical level. Hip circumference was measured at the level of the greatest posterior protuberance. Waist-to-hip ratio was calculated as WC divided by HC.

Blood sampling and analysis

At baseline and 6 months, blood samples were collected after overnight fasting (at least 12 hr). The blood biochemical variables including blood glucose, total protein (TP), albumin (ALB), total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), blood urea nitrogen (BUN), iron, unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), folic acid, vitamin B-12, glycosylated hemoglobin (HbA1c), and C-reactive protein (CRP) and blood routine were measured in the Biochemistry Division of Chinese PLA General Hospital.

We measured the blood level of immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin E (IgE), immunoglobulin light-strand KAP (Ig light-strand KAP), immunoglobulin light-strand LAM (Ig light-strand LAM) and complement 3 (C3) and complement 4 (C4) for humoral immunity and CD^{4+}, CD^{8+}, blood white cells, lymphocytes, neutrophils, lymphomonocytes, basophilic leukocytes, and eosinophile granulocytes for cellular immunity at baseline and at 6 months in the Clinical Chemistry Examination Division of Chinese PLA General Hospital. CD^{4+}/CD^{8+} ratio was calculated.

Blood sampling for determination of the blood biochemical variables, immune functions and anthropometric measurements was done on the same day.

Statistical analysis

Data were expressed as means ± SD for measurement data or means ± SE for enumeration data. Differences of values in the same group between the baseline and 6

months of the study were examined using the paired *t*-test. Differences in two groups for the average percentages of common infection were compared by the Student's *t*-test or chi-square test. To improve the accuracy of the estimates, a general linear model, including baseline values as covariates, a analysis of covariance (ANCOVA) was performed to compared differences for measurement data between placebo and supplementation group at 6 months. All statistical analyses were performed using SPSS Version 13.0 for Windows. Statistical significance was determined at α=0.05.

RESULTS

Characteristics of patients

A total of 14 patients (7 in each group) were excluded from the study because of various reasons. In the supplementation group, one had traffic accident, one suffered from cerebral infarction, one suffered from cancer, two went aboard and two were not willing to consume the micronutrient. In the placebo group, one suffered from heart infarction, two were not willing to consume the placebo and four were withdrawn because of their loss of contact. The rate of dropout was 7.1%, and data for 182 DM patients were finally analyzed. The clinical characteristics of patients in the supplementation (n=90) and placebo (n=92) group were listed in Tables 1 and 2. Both groups did not differ in age, sex, anthropometric measurements, duration of DM, cigarette smoking, alcohol drinking and medication at baseline or at 6 months.

Energy and nutrients intake and physical activity time

The supplementation and placebo group did not differ in

Table 1. The clinical characteristics of diabetic subjects receiving micronutrient supplementation or placebo at baseline and at 6-month follow-up

	Placebo		Supplementation	
	Baseline	6 months	Baseline	6 months
Patients (M/F)[†]	97 (36/61)	90 (34/56)	99 (37/62)	92 (36/56)
Age (year)	63.1±9.88	-	62.9±9.01	-
Height (cm)	160±7.77	160±7.85	161±6.93	161±7.00
Body weight (kg)	64.1±10.8	64.1±10.7	62.9±9.70	62.9±9.65
Body mass index (kg/m^2)	24.8±3.51	24.9±3.48	24.0±3.08	24.±3.09
Waist circumference (cm)	87.3±9.24	87.4±9.25	85.7±7.76	85.5±7.66
Hip circumference (cm)	96.9±7.74	97.0±7.80	95.9±6.25	95.9±6.11
Waist-to-hip ratio	0.90±0.06	0.90±0.06	0.89±0.05	0.89±0.05
Duration of diabetes (year)	5.67±1.17	5.82±1.13	5.84±1.28	5.74±1.25
Alcohol drinking (M/F)	28 (16/12)	27 (16/11)	26 (16/10)	26 (16/10)
Cigaret smoking (M/F)	16 (14/2)	16 (14/2)	15 (12/3)	15 (12/3)
Sulfonylureas (M/F)	17 (6/11)	15 (6/9)	16 (6/10)	15 (6/9)
Biguanides (M/F)	31 (8/23)	30 (8/22)	32 (11/21)	31 (11/20)
Glucosidase inhibitors (M/F)	22 (9/13)	21 (9/12)	25 (11/14)	24 (11/13)
Diet control only (M/F)	27 (13/14)	24 (11/13)	26 (16/10)	22 (12/10)

[†]M: number of males; F: number of females

Table 2. Intake of energy, macro and micronutrients, energy percentage of macronutrients and daily physical activity time in diabetic patients receiving micronutrient supplementation or placebo

	Placebo (n=90)		Supplementation (n=92)	
	Baseline	Average of 6 months	Baseline	Average of 6 months
Energy (kcal)	1655±252	1736±226	1678±259	1741±212
Protein (g)	70.5±16.11	70.7±13.1	73.2±17.7	72.9±14.5
Fat (g)	51.0±9.29	51.9±6.97	52.5±10.3	52.7±4.80
Carbohydrate (g)	240±53.2	249±41.9	236±47.0	249±47.3
Protein (%)	16.9±2.32	16.3±1.95	17.4±2.64	16.7±2.40
Fat (%)	28.0±4.71	27.3±3.62	28.3±4.50	27.7±3.10
Carbohydrate (%)	58.0±7.58	57.7±5.01	56.3±5.95	57.0±5.70
Vitamin A (μgRE)	586±134	579±67.3	588±157	615±249
Carotene (μg)	2200±252	2095±170	2154±265	2118±214
Vitamin E (mg)	33.4±8.19	35.7±5.47	34.3±8.10	32.9±9.90
Vitamin B-1 (mg)	1.07±0.28	1.05±0.23	1.01±0.27	1.10±0.20
Vitamin B-2 (mg)	1.11±0.26	1.11±0.25	1.10±0.24	1.10±0.20
Niacin (mg)	15.0±4.09	15.1±3.03	15.1±4.93	15.6±3.70
Vitamin C (mg)	159±66.3	147±40.0	146±59.5	142±49.1
Calcium (mg)	669±193	665±146	690±189	671±171
Phosphorus (mg)	2388±895	2273±721	2382±564	2268±717
Potassium (mg)	2086±487	2077±482	2020±562	2072±490
Sodium (mg)	2579±307	2546±200	2590±275	2246±910
Magnesium (mg)	273±61.2	291±24.8	273±65.5	288±58.4
Iron (mg)	17.7±3.78	18.1±3.13	17.2±3.37	17.8±2.90
Zinc (mg)	9.87±2.38	9.96±1.41	9.68±1.91	9.60±1.80
Selenium (μg)	37.2±8.89	37.5±6.74	39.2±9.56	38.6±7.20
physical activity time (min/d)	251.8±79.1	253±41.5	251±64.5	333±143

terms of intake of daily energy or macro- or micronutrients or physical activity time at baseline, and at 1 to 6 months. There were no significant differences in values from baseline to 6 months for each of the groups (Table 2).

Blood biochemical variables

At 6 months, all of the blood biochemical variables in the placebo group did not differ compared to the baseline, however, in the supplementation group, the blood concentrations of Fe and folic acid were significantly higher and UIBC level significantly lower compared to the baseline. On the other hand, compared to the placebo, the blood concentrations of total protein, Fe, folic acid and hemoglobin increased and UIBC levels decreased at 6 months. Furthermore, compared to the placebo group, the increasing extent for the blood concentration of total protein, iron, folic acid and hemoglobin and the decreasing extent for UIBC in the supplementation group were greater (Table 3).

Immune functions

Between the placebo and supplementation group, the indicators of immune function were similar at baseline. At 6 months, compared to the placebo group, the blood concentrations of IgE, CD^{4+}, CD^{4+}/CD^{8+}, WBC, lymphocyte counts, basophilic leukocyte increased and CD^{8+} count decreased in the supplementation group. Furthermore, the increasing extent for IgE, CD^{4+}, CD^{4+}/CD^{8+}, WBC, lym-

Table 3. Blood biochemical variables of patients with diabetes receiving micronutrient supplementation or placebo at baseline and 6 months

	Placebo			Supplementation		
	Baseline (n=97)	6 months (n=90)	⊿†	Baseline (n=99)	6 months (n=92)	⊿†
Glucose (mmol/L)	7.08±1.91	7.28±2.01	0.19±1.65	6.89±1.73	6.81±1.36	0.14±0.86
Total protein(g/L)	73.2±4.00	70.0±4.19	-3.19±4.93	71.7±4.05	72.0±4.18*	0.28±6.68*
Albumin(g/L)	46.1±2.46	44.7±1.99	-1.44±3.25	45.61±2.40	45.1±2.28	-0.52±2.79
Cr (mmol/L)	62.9±12.4	64.3±14.0	1.31±6.18	63.0±13.4	63.9±14.9	1.21±5.95
BUN (mmol/L)	5.88±1.31	5.68±1.25	-0.20±1.20	5.97±1.26	5.64±1.18	-0.33±1.17
TG (mmol/L)	1.61±0.73	1.51±0.56	-0.09±0.64	1.58±0.81	1.47±0.67	-0.11±0.59
Total CHO (mmol/L)	5.05±0.83	5.34±1.06	0.28±0.91	4.99±0.87	5.08±0.93	0.10±0.65
HDL-C (mmol/L)	1.33±0.32	1.40±0.28	0.07±0.32	1.29±0.33	1.37±0.33	0.08±0.18
LDL-C (mmol/L)	2.93±0.62	2.76±0.71	-0.14±0.63	2.95±0.71	2.59±0.67	-0.36±0.57
Iron (μmol/L)	16.3±4.59	16.8±5.12	0.52±5.47	16.7±4.93	18.3±5.48*,**	1.63±5.63*
UIBC (μmol/L)	43.8±9.32	41.8±11.1	-2.03±9.49	45.6±11.8	39.2±10.6*,**	-6.39±9.63*
TIBC (μmol/L)	60.1±8.15	58.4±9.54	-1.58±7.58	60.9±8.49	57.8±8.96	-3.10±8.75
Folic acid (ng/ml)	10.3±4.10	11.1±4.10	0.59±2.87	9.42±3.27	16.6±4.10*,**	7.14±4.42*
Vitamin B-12 (pg/ml)	744±469	757±442	13.4±301	659±410	779±493	118±361
HbA1c (%)	6.69±1.20	6.68±1.33	-0.01±0.80	6.71±1.28	6.59±1.54	-0.12±0.96
CRP (mg/dl)	0.32±0.07	0.32±0.07	-0.01±0.06	0.34±0.14	0.34±0.14	0.00±0.14
Hemoglobin(g/L)	141±14.5	144±15.3	1.92±8.92	141±15.1	145±12.8*,**	3.97±0.52*
Hematocrit(L/L)	0.43±0.04	0.44±0.04	0.01±0.03	0.43±0.04	0.44±0.04	0.01±0.03

†6 months minus baseline. *p<0.05 compared to placebo control, **p<0.05 compared to baseline in the same group.

Table 4. Changes in humoral and cellular immunity in patients with diabetes receiving micronutrient supplementation or placebo at baseline and at 6 months

	Placebo			Supplement		
	Baseline (n=97)	6 months (n=90)	⊿†	Baseline (n=99)	6 months (n=92)	⊿†
IgA (mg/dl)	291.5±107	295±111	3.48±64.6	289±122	295±138	5.53±63.2
IgM (mg/dl)	93.1±46.1	101±47.9	8.14±22.4	106±69.8	116±82.7	9.73±38.8
IgG (mg/dl)	1389±281	1456±298	67.0±263	1354±312	1459±294	104±344
IgE (IU/ml)	93.2±139	79.4±127	-13.9±43.9	103±210	97.0±180*	-5.53±82.9*
Ig light-strand KAP (mg/dl)	343±70.7	345±76.1	1.60±40.6	345±71.7	351±78.1	4.80±54.3
Ig light-strand LAM (mg/dl)	192±44.3	198±43.4	4.98±30.7	95.7±53.7	200±51.3	4.32±26.8
C3 (mg/dl)	125±19.8	128±22.9	3.16±20.3	128±22.0	132±21.8	3.76±21.0
C4 (mg/dl)	29.9±9.31	29.7±8.89	-0.18±5.63	28.6±8.80	28.6±7.55	0.01±7.80
CD^{4+} (%)	0.38±0.08	0.37±0.09	-0.02±0.07	0.36±0.09	0.38±0.10*	0.02±0.05*
CD^{8+} (%)	0.27±0.09	0.27±0.09	0.01±0.04	0.28±0.10	0.27±0.10*	-0.02±0.04*
CD^{4+}/CD^{8+}	1.60±0.69	1.53±0.74	-0.07±0.40	1.46±0.68	1.68±0.84*	0.22±0.35*
WBC ($\times 10^9$/L)	6.32±1.75	6.31±1.67	0.00±0.90	6.35±1.36	6.38±1.95*	0.04±1.70*
Neutrophil (%)	58.4±6.66	59.9±7.52	1.41±5.53	58.0±7.79	59.9±8.41	1.66±7.52
Lymphocyte (%)	34.6±6.87	32.9±7.39	-1.70±6.39	33.4±7.33	33.7±7.09*	0.19±6.18*
Lymphomonocyte (%)	5.40±1.19	5.23±5.86	-0.14±5.79	5.30±1.15	5.28±6.66	-0.03±6.71
Basophilic leukocyte (%)	0.42±0.24	0.52±0.43	0.10±0.43	0.39±0.28	0.57±0.58*	0.18±0.50*
Eosinophile granulocyte (%)	2.12±1.28	1.95±1.18	-0.14±1.08	2.57±1.75	2.25±1.78	-0.22±1.34

†6 months minus baseline. *p<0.05 compared to placebo control.

Table 5. The average percentages of common infections in diabetic patients receiving micronutrient supplementation or placebo at baseline and at 6 months

	Baseline	1 month	2 month	3 month	4 month	5 month	6 month	Mean±SD‡
Placebo (n=90)								
Common cold	22.2±4.38	28.9±4.78	23.3±4.46	22.2±4.38	25.6±4.60	23.3±4.46	21.1±4.30	24.1±4.51
Fever	22.2±4.38	25.6±4.60	22.2±4.38	20.0±4.22	24.4±4.53	25.6±4.60	21.1±4.30	23.8±4.49
Temperature of fever (℃)†	38.2±0.41	38.4±0.66	38.1±0.54	38.3±0.48	38.0±0.36	38.2±0.58	38.2±0.64	38.2±0.50
Days of fever (days)†	2.40±0.50	2.36±0.92	2.33±0.69	2.47±1.07	2.38±0.50	2.28±0.93	2.21±0.64	2.30±0.65
Sore throat	13.3±3.58	14.4±3.71	13.3±3.58	11.1±3.31	16.7±3.93	14.4±3.71	13.3±3.58	13.9±3.65
Whitlow	21.1±4.30	28.9±4.78	24.4±4.53	28.9±4.78	23.3±4.46	22.2±4.38	23.6±4.48	25.2±4.58
Dermapostasis	24.4±4.53	25.6±4.60	24.4±4.53	23.3±4.46	22.2±4.38	16.7±3.93	15.6±3.82	21.3±4.32
Anthracoma, furuncle	13.3±3.58	12.2±3.45	10.0±3.16	11.1±3.31	14.4±3.71	13.3±3.58	13.3±3.58	12.4±3.47
Pyelonephritis, cystitis	15.6±3.82	14.4±3.71	13.3±3.58	14.4±3.71	13.3±3.58	12.2±3.45	12.2±3.45	13.3±3.58
Vaginitis, urinary tract infection	17.8±4.03	23.3±4.46	21.1±4.30	18.9±4.13	23.3±4.46	19.1±4.14	15.6±3.82	20.2±4.23
Cholecystitis, gallstone	21.1±4.30	22.2±4.38	19.1±4.14	18.9±4.13	23.3±4.46	23.6±4.48	23.3±4.46	21.8±4.35
Gingivitis	41.1±5.19	34.4±5.01	33.3±4.97	34.4±5.01	33.3±4.97	34.4±5.01	33.3±4.97	33.9±4.99
Dental ulcer	33.3±4.97	33.3±4.97	35.6±5.05	34.4±5.01	34.4±5.01	31.1±4.88	26.7±4.66	32.6±4.94
Supplementation (n=92)								
Common cold	22.8±4.38	19.6±4.14	17.4±3.95	13.0±3.51	19.6±4.14	14.1±3.63	10.9±3.25	15.8±3.80*
Fever	22.8±4.38	20.7±4.22	17.4±3.95	13.0±3.51	18.5±4.05	13.0±3.51*	6.52±2.57*	14.9±3.71*
Temperature of fever (℃)†	38.2±0.54	38.1±0.49	37.8±0.64	37.8±0.52*	37.9±0.07	37.7±0.24*	37.4±0.29*	37.8±0.40*
Days of fever (days)†	2.39±0.85	2.22±0.81	2.06±0.56	1.75±0.45*	1.59±0.51*	1.67±0.49*	1.50±0.55*	1.82±0.63*
Sore throat	12.0±3.38	12.0±3.38	10.9±3.25	8.70±2.94	14.1±3.63	13.0±3.51	9.78±3.10	11.4±3.32
Whitlow	21.7±4.30	22.8±4.38	19.6±4.14	17.4±3.95	18.5±4.05	15.2±3.74	13.0±3.51	17.8±3.98*
Dermapostasis	22.8±4.38	22.8±4.38	19.6±4.14	13.0±3.51	15.2±3.74	8.70±2.94	7.61±2.76	14.5±3.67*
Anthracoma, furuncle	14.1±3.63	13.0±3.51	12.0±3.38	12.0±3.38	13.0±3.51	13.0±3.51	12.0±3.38	12.5±3.45
Pyelonephritis, cystitis	16.3±3.85	15.2±3.74	13.0±3.51	12.0±3.38	13.0±3.51	12.0±3.38	12.0±3.38	12.9±3.49
Vaginitis, urinary tract infection	17.4±3.95	15.2±3.74	13.0±3.51	7.61±2.76	14.1±3.63	8.70±2.94*	5.43±2.36*	10.7±3.22*
Cholecystitis, gallstone	27.2±4.64	26.1±4.58	21.7±4.30	20.7±4.22	20.7±4.22	18.5±4.05	16.3±3.85	20.7±4.22
Gingivitis	42.4±5.15	34.8±4.97	31.5±4.84	22.8±4.38	22.8±4.38	17.4±3.95*	15.2±3.74*	24.1±4.46*
Dental ulcer	34.8±4.97	30.4±4.80	28.3±4.69	22.8±4.38*	22.8±4.38*	16.3±3.85	13.0±3.51	22.3±4.34*

†the absolute values, *t*-test was used, others were *chi-square* test. ‡Average of 6 months. *$p<0.05$ compared to placebo control.

phocyte counts and basophilic leukocyte, and the decreasing extent for CD^{8+} count from baseline to 6 months were greater in the supplementation group than in the placebo group (Table 4).

Common infections

No one was hospitalized for medical treatment because of severe infections at baseline and during the study. The mean incidence of upper respiratory infection was lower in the supplementation than in the placebo group during the study. The incidence of fever was less at months 5 and 6 in the supplementation than in the placebo group. Body temperature and duration of fever during infection were lower and shorter, respectively, in the supplementation than in the placebo group. The mean incidence of whitlow, dermapostasis, vaginitis, urinary tract infection, gingivitis and dental ulcers was lower in the supplementation than in the placebo group during the study (Table 5).

DISCUSSION

Patients with DM have infections more often than those without DM. Despite recent advances in the management of DM and intense glucose control reducing vascular complications in DM,[11] DM patients still present with increased susceptibility to various infections.[1,2,4,12,13] Although the exact mechanism of this increased incidence is less well established, studies of the immune cells of DM patients have demonstrated significant defects in both humoral and cellular function.[4,13] Theoretically, protein and energy, micronutrients such as Zn, Se, Fe, Cu, vitamins A, C, E, and D and folic acid are important for normal immune function.[5-8] Deficiencies in these micronutrients may impair immunity[7,8] and increase susceptibility to infections. Thus in the present study, our aim was to confirm whether supplementation with micronutrients could improve immune function and decrease the incidence of common infections in DM.

Although the effect of a single micronutrient on specific infectious disease is unclear, an adequate intake of vitamins B-12, C, E, folate, selenium, zinc, copper, and iron was found to contribute to the maintenance of an effective immune response and to counteracting infections. For example, a meta-analysis of large clinical trials showed that vitamin A supplementation reduced the severity of diarrhea disease and pneumonia.[14,15] A supplementation, together with zinc, was shown to improve the mucosal epithelial barriers and diminish the incidence of dermapostasis.[16] The antioxidant nutrients: vitamins C and E contributed to preventing the infection of upper respiratory system.[17,18] Vitamin D ($1,25\text{-}(OH)_2D_3$) deficiency is correlated with a higher susceptibility to infections due to impaired renal disease.[19] High intakes of folic acid (>400 μg/day) might be beneficial for preventing infections of the digestive system, as well as dental ulcer.[20] Selenium, copper, and zinc were involved in antioxidant defense as cofactors of enzymes such as GSPX and SOD, and iron could prevent anemia, they might fight against the infection of many systems.[21-24] In the present clinical trial, we observed that supplementation of combined micronutrient reduced the incidence of common infection involved in upper respiratory, skin, urinary and oral cavity systems in type 2 DM outpatients.

These might due to the immune function of subjects in supplementation group being improved. Micronutrient deficiency suppresses immune functions by affecting the innate T-cell-mediated immune response and adaptive antibody response, and leads to dysregulation of the balanced host response.[25-28] Although some reports indicated that alterations of CD^{4+}/CD^{8+} ratio during the evolution and progression of DM had no relationship to infections detected,[29,30] and no agreement has been reached as to whether the number and function of T and B cells in patients with DM increased, decreased, or remain unchanged as compared to controls.[31] Moreover, previous reports had found that DM patients were more likely than non-DM patients to be deficient in one or more micronutrients. Deficiency or insufficiency of one or more micronutrients linked to immunity might impair immunity.[7] For example, enhancement of the percentage of CD^{4+} cells was observed after Zn therapy in elderly patients with diabetes mellitus.[32] One study of diabetic patients showed decreased functions (chemotaxis, phagocytosis, killing) of diabetic polymorphonuclear cells and diabetic monocytes or macrophages as compared with control subjects.[4] Vitamin E supplementation decreased parameters of oxidative stress, proliferation of lymphocytes, and improved the CD^{4+}/CD^{8+} ratio.[33] Vitamin A plays an important role in both cell-mediated and humoral antibody response and acts as an anti-inflammatory substance (Th2 response).[34] Folate deficiency also increased the ratio of CD^{4+} to CD^{8+} T cells due to a marked reduction in CD^{8+} cell proliferation.[35] Selenium deficiency decreased immunoglobulin titers of IgM and IgG, and increased CD^{4+} T cells and decreased CD^{8+} and CD^{4+}/CD^{8+}.[36] Also the ratio of T lymphocytes (CD^{4+} to CD^{8+} cells) in blood reduced in iron deficiency.[37]

In the present study, we found an increased count of total lymphocytes and CD^{4+}, reduced count of CD^{8+}, and increased ratio of CD^{4+}/CD^{8+} in DM patients with micronutrient supplementation. These findings were associated with decreased incidence of common infection and attenuated symptoms and signs during common infections. Therefore, micronutrient supplementation may be beneficial for type 2 DM patients to prevent or reduce common infections.

However, the present results showed no improvement in IgA, IgM, IgG and C3 and C4 levels except IgE in DM patients with micronutrient supplementation. This finding implied that nutritional supplementation of micronutrients did not favor humoral immunity in type 2 DM patients. Humoral immunity refers to the branch of immunity that is mediated by secreted antibodies produced in the B cells. Humoral immunity is particularly effective against extracellular pathogens. How cellular immunity was improved in DM patients with micronutrient supplementation is still unclear, although we found increased count of lymphocytes and CD^{4+}. Future studies are needed to resolve these issues.

Energy-protein malnutrition induces both dysfunction of humoral and cellular immunity.[38] Nutrient insufficiency was defined as intake below the recommended nutrient intake (RNI) as revised by the Chinese Nutrition Society in 2000. The RNI is for normal Chinese people with age >50 years. There was no standard cut-off point for micronutrient insufficiency or deficiency for type 2 DM patients in China, so we chose the RNI. To understand the dietary intake of energy, macro- and micronutrients in our DM subjects, we surveyed diet at baseline and 6 times during the study. The daily intake of energy and macronutrients in both supplement and placebo groups almost met the RNI for energy and macronutrients suggested by the Chinese Nutrition Society or the American Diabetes Association.[39,40] Moreover, anthropometric measurements, blood biochemical variables and hemoglobin suggested no symptoms or signs of energy-protein malnutrition in either group. Therefore, we suggest no impairment of immune function in these patients because of energy-protein malnutrition. On the other hand, our aim is to observe the effect of supplying micronutrients; so, micronutrients intake from diet in two groups should be comparable. From Table 2, the supplement and placebo groups did not differ in intake of macro- or micronutrients at baseline and at 1 to 6 months, to ensure comparability for analysis.

In our study, some blood biochemical variables such as glucose, lipids, albumin etc. did not differ between the two groups at baseline or at 6 months (Table 3). Because the enrollment criteria used were strict,[41] and medication for diabetes did not change during the study, and we did not investigate the relationship between glycaemic control and the incidence of common infections. We are not sure that only micronutrient supplementation might affect blood biochemical variables. Also, the relationship between metabolic control and development of long-term complications of DM was one of the most contentious issues in medicine.[42] Future mechanism studies will be needed.

In conclusion, micronutrient supplementation in type 2 DM could increase lymphocyte and CD^{4+} count and reduce the incidence of common infections. This supplementation could be of great clinical significance for DM

patients to improve their quality of life and control infection because micronutrient supplementation is affordable, safe and convenient.

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AUTHOR DISCLOSURES

The authors have no conflicts of interest.

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Original Article

Micronutrients decrease incidence of common infections in type 2 diabetes outpatients

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补充微量营养素减少 2 型糖尿病患者一般感染发生率

按随机双盲安慰剂对照的研究方法，分别给予 196 例 2 型糖尿病患者微量营养素制剂(n=97)和安慰剂(n=99)，连续服用 6 个月。推荐每日膳食摄入量及运动量。于研究开始前和结束后进行人体测量、血液生化指标及一般感染发生率检查。研究前 1 个月及研究期间每月进行随访，收集饮食和运动记录表、感染登记表。研究结束后微量营养素补充剂组与安慰剂组比较，平均血清总蛋白浓度、血清铁和叶酸水平以及血红蛋白浓度、IgE 水平、$CD4^+$计数、$CD4^+/CD8^+$比例，白细胞计数、淋巴细胞计数、嗜碱性粒细胞计数显著升高，不饱和铁结合力（UIBC）以及 $CD8^+$计数显著下降，而 IgA、IgM、IgG 和补体 C3 和 C4 水平两组间无显著性差异。与安慰剂组比较，在研究期间微量营养素补充剂组的上呼吸道感染、咽痛、皮肤脓肿与破溃、阴道炎、泌尿系统感染、牙龈炎、口腔溃疡的平均发生率均显著降低，且发热时体温和持续时间均有明显改善。因此，适量补充微量营养素可提高 2 型糖尿病患者免疫功能，减少一般感染的发生率。

关键字：2 型糖尿病、微量营养素、感染、免疫功能、营养