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The role of *Enterobacteria*, TNF-α, IL-6, and IL-10 in the development of myocardial ischemia with type 2 diabetes mellitus

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Abstract

This study is to observe the distribution of intestinal flora and the changes of inflammatory factors in elderly patients with myocardial ischemia complicated with type 2 diabetes mellitus. A total of 106 elderly patients with myocardial ischemia complicated with type 2 diabetes mellitus (complicated group), 106 elderly patients with simple type 2 diabetes mellitus (diabetic group), and 106 healthy elderly people (control group) were selected. The fasting blood glucose (FBG), 1-h postprandial blood glucose (1hPG), 2-h postprandial blood glucose (2hPG), 3-h postprandial blood glucose (3hPG), and hemoglobin A1c (HbA1c) in complicated group and the diabetic group were higher than those in the control group ($P<0.05$ or $P<0.01$). The duration of diabetes, FBG, 3hPG, and HbA1c in the complicated group were higher than those in the diabetic group, while the 2hPG was lower than that in the diabetic group ($P<0.05$). Compared with control group, the number of *Enterobacteria* in the diabetic group and complicated group was increased, while the numbers of *Bacteroides, Bifidobacteria*, and *Lactobacillus* were decreased ($P<0.05$ or $P<0.01$). Compared with the diabetic group, the number of *Enterobacteria* in complicated group was increased, while the numbers of *Bacteroides, Bifidobacteria*, and *Lactobacillus* were decreased ($P<0.05$ or $P<0.01$). Compared with control group, the levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 beta (IL-1β), and C-reactive protein (CRP) decreased in the diabetic group and complicated group, and the lowest in the complicated group. Conversely, the levels of interleukin 10 (IL-10) and interleukin 12 (IL-2) increased in the diabetic group and complicated group, and the highest in the complicated group ($P<0.05$ or $P<0.01$). Multiple logistic regression analysis showed that the duration of diabetes, HbA1c, *Enterobacteria*, TNF-α, IL-6, and IL-10 were the influencing factors of myocardial ischemia complicated with type 2 diabetes mellitus ($P<0.05$ or $P<0.01$). In conclusion, in the elderly patients with myocardial ischemia complicated with type 2 diabetes mellitus, the number of intestinal probiotics and the level of anti-inflammatory factors decreased, and the number of pathogenic bacteria and the level of inflammatory factors increased. *Enterobacteria*, TNF-α, IL-6, and IL-10 may play an important role in the development of myocardial ischemia in type 2 diabetes mellitus.

Keywords

elderly patients, inflammatory factor, intestinal flora, myocardial ischemia, type 2 diabetes mellitus

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Diabetes is a major risk factor for cardiovascular diseases, and often leads to serious cardiovascular events (peripheral arterial disease, myocardial infarction, sudden death, etc.), and coronary heart disease accounts for about 75% of the deaths of diabetes.[1,2] In diabetic patients, the incidence of asymptomatic myocardial ischemia (the patient has no subjective

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chest pain and chest distress but there is objective evidence for myocardial ischemia) significantly increased.[3] Relevant data show that[4] the incidence of painless myocardial infarction in diabetic patients is the same as that of non-diabetic patients. In the electrocardiographic exercise test with the same ST-T wave changes, the incidence of angina pectoris in diabetic patients was three times lower than that in non-diabetic patients, but the incidence of myocardial perfusion abnormalities in diabetic patients was the same as non-diabetic patients with coronary heart disease.[5] Intestinal microbiota has attracted much attention in recent years. *Bifidobacteria, Lactobacillus, Enterococcus, Bacteroides*, and *Enterobacteria* are the most common bacteria in the intestine. *Bifidobacteria* and *Lactobacillus* are probiotics, which have the effect of reducing intestinal pH and inhibiting the growth of pathogenic bacteria.[6,7] *Bacteroides* have an important role in maintaining the stability of the intestinal flora. *Enterobacteria* and *Enterococcus* are the most common pathogens in the intestine that threaten human health. When various causes lead to the decrease of probiotics and the increase of pathogenic bacteria, intestinal microecological environment will be destroyed and will lead to intestinal dysbacteriosis.[8,9] Intestinal flora and intestinal microecology are closely related to digestion, absorption, movement, secretion, regulation, and reflex of the enteric nervous system. If the composition of the intestinal microflora changes, it will not only induce a variety of gastrointestinal diseases but also induce chronic diseases such as obesity, cardiovascular disease, diabetes, and coronary heart disease.[10] In this study, we investigated the distribution of intestinal microflora and the level of inflammatory factors in elderly patients with myocardial ischemia complicated with type 2 diabetes mellitus and explored the relationship between them and myocardial ischemia complicated with type 2 diabetes mellitus, in order to further understand the pathogenesis of the disease.

Objects and methods

Research objects

A total of 106 patients with myocardial ischemia complicated with type 2 diabetes mellitus (complicated group) from August 2016 to December 2017 in our hospital, including 62 males and 44 females, aged 61–76 years, with an average age of (65.27 ± 2.84) years old were selected. There were 106 patients with simple type 2 diabetes mellitus (diabetic group), including 61 males and 45 females, aged 60–75 years, with an average age of (65.16 ± 3.02) years. In addition, 106 healthy subjects were selected as control group, including 61 males and 45 females, aged 61–74 years, with an average age of (65.08 ± 2.75) years. If the patients met the 1999 World Health Organization (WHO) diabetes diagnosis and classification criteria, it was diagnosed as type 2 diabetes mellitus. Resting electrocardiogram or treadmill exercise test, 24-h dynamic electrocardiogram, and color Doppler echocardiography were performed, and asymptomatic myocardial ischemia could be diagnosed if two feces of the three tests were in accordance with the positive criteria. Patients with other gastrointestinal and other organic lesions of the digestive tract; with cholecystitis, gallstones, pancreatic diseases, connective tissue disease, multiple sclerosis, and other systemic diseases that may affect gastric motility; with the presence of liver and kidney dysfunction, electrolytes disorders, and acute complications were excluded. All participants in this research signed informed consent for their participation.

Research methods

General information and biochemical indicators collection

The gender, age, duration of diabetes, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded, and body mass index (BMI) was calculated. After 10–12 h of fasting, the venous blood was extracted in the early morning to detect fasting blood glucose (FBG), 1-h postprandial blood glucose (1hPG), 2-h postprandial blood glucose (2hPG), 3-h postprandial blood glucose (3hPG), and hemoglobin A1c (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The FBG, 1-h PG, 2-h PG, and 3-h PG were determined by 75 g oral glucose tolerance test (OGTT). HbA1c was determined by high-performance liquid chromatography.

Intestinal flora detection

A sterilized cotton swab was used to take about 8 to 10 g of fresh feces for the first time on the day to make a uniform suspension. *Enterobacteria, Bacteroides*, and *Bifidobacteria* were inoculated

with the injection method; *Enterococcus* and *Lactobacillus* were inoculated into their own special culture medium.[11] The anaerobic bacteria culture medium was incubated in a glove-type anaerobic chamber for 48 h, and aerobic bacteria were placed in a conventional constant-temperature incubator for 24 h. Culture media for *Enterobacteria, Bacteroides, Bifidobacteria, Enterococcus*, and *Lactobacillus* were purchased from Merck (Darmstadt, Germany). Culture media with 30–300 colonies were selected for counting. The average of the same dilution was taken, and the specific formula for colony-forming units (CFU) = the average number of colonies × dilution × 50. All culture and anaerobic bacteria routine biochemical results were compared with international standard strains as quality control. The identification of flora species was performed according to the morphology of the bacteria, the characteristics of colonies, and the characteristics of colony Gram stain. The KOH method was used to distinguish between gram-negative and gram-negative colonies.[12]

Inflammatory factor detection

About 3 mL venous blood in the morning was taken, centrifuged at 2000 r/min for 10 min, and the separated serum was placed in a refrigerator at −80°C. Enzyme-linked immunosorbent assay (ELISA) was used to detect tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 beta (IL-1β), interleukin 10 (IL-10), and interleukin 12 (IL-2). The kit was purchased from Shanghai Kanglang Biotechnology Co., Ltd. and was performed according to the operating instructions. The C-reactive protein (CRP) was measured using an automatic biochemical analyzer.

Statistical analysis

SPSS 21.0 software was used for statistical analysis. Measured data were expressed as $\bar{x} \pm s$, differences among groups were compared with one-way analysis of variance, and least significant difference (LSD) t test was used for the comparison between two groups. Logistic regression was used to analyze the risk factors of myocardial ischemia complicated with type 2 diabetes mellitus. The P value <0.05 indicates that the difference was statistically significant.

Results

Comparison of general data and biochemical indicators of each group

FBG, 1hPG, 2hPG, 3hPG, and HbA1c were higher in the complicated group and diabetic group than in the control group ($P<0.05$ or $P<0.01$). The duration of diabetes, FBG, 3hPG, and HbA1c in complicated group were higher than those in the diabetic group. The 2hPG in complicated group was lower than that in the diabetic group ($P<0.05$), as shown in Table 1.

Comparison of intestinal flora in each group

Compared with control group, the number of *Enterobacteria* in the diabetic group and complicated group was increased, while the numbers of *Bacteroides, Bifidobacteria*, and *Lactobacillus* were decreased ($P<0.05$ or $P<0.01$). Compared with the diabetic group, the number of *Enterobacteria* in complicated group was increased, while the numbers of *Bacteroides, Bifidobacteria*, and *Lactobacillus* were decreased ($P<0.05$ or $P<0.01$). There was no significant difference in the number of *Enterococcus* between the groups ($P>0.05$), as shown in Table 2.

Comparison of levels of inflammatory factors in each group

Compared with control group, the levels of TNF-α, IL-6, IL-1β, and CRP decreased in the diabetic group and complicated group, and the lowest in the complicated group. Conversely, the levels of IL-10 and IL-2 increased in the diabetic group and complicated group, and the highest in the complicated group ($P<0.05$ or $P<0.01$), as shown in Table 3.

Multivariate logistic regression analysis of risk factors for myocardial ischemia complicated with type 2 diabetes mellitus

Myocardial ischemia complicated with type 2 diabetes mellitus was used as a dependent variable, and multiple logistic regression analysis was performed with age, the duration of diabetes, blood glucose, blood lipids, HbA1c, intestinal flora, and inflammatory factors as independent variables. The results showed that the duration of diabetes, HbA1c, *Enterobacteria*, TNF-α, IL-6, and IL-10

Table 1. Comparison of general data and biochemical indicators of each group ($\bar{x} \pm s$).

Group	Case (male/female)	Age (years)	Duration of diabetes (years)	BMI (kg/m^2)	SBP (mm Hg)
Control group	106 (61/45)	65.08 ± 2.75	0	24.41 ± 1.36	114.36 ± 9.52
Diabetic group	106 (61/45)	65.16 ± 3.02	9.43 ± 2.76	24.78 ± 1.44	115.12 ± 7.85
Complicated group	106 (62/44v	65.27 ± 2.84	13.14 ± 3.85$^{\triangle}$	24.95 ± 1.29	113.08 ± 8.66
Group	DBP (mm Hg)	FBG (mmol/L)	1hPG (mmol/L)	2hPG (mmol/L)	3hPG (mmol/L)
Control group	74.21 ± 8.32	4.22 ± 0.45	6.65 ± 0.51	5.28 ± 0.43	4.21 ± 0.87
Diabetic group	77.62 ± 7.46	7.76 ± 1.31*	10.68 ± 2.77$^{\#}$	13.32 ± 2.74$^{\#}$	10.54 ± 2.35$^{\#}$
Complicated group	76.44 ± 6.78	9.13 ± 1.49$^{\#\triangle}$	10.45 ± 1.61$^{\#}$	11.77 ± 1.56$^{\#\triangle}$	12.15 ± 2.11$^{\#\triangle}$
Group	HbA1c (%)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Control group	5.28 ± 0.45	5.03 ± 0.65	1.64 ± 0.76	2.76 ± 0.08	1.53 ± 0.25
Diabetic group	9.55 ± 1.69$^{\#}$	5.12 ± 0.61	1.61 ± 0.48	2.79 ± 0.09	1.55 ± 0.36
Complicated group	11.41 ± 1.83$^{\#\triangle}$	5.27 ± 0.62	1.73 ± 0.32	2.86 ± 0.05	1.52 ± 0.31

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; 1hPG: 1-h postprandial blood glucose; 2hPG: 2-h postprandial blood glucose; 3hPG: 3-h postprandial blood glucose; HbA1c: hemoglobin A1c; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.
Compared with control group, $^{*}P < 0.05$; $^{\#}P < 0.01$; compared with diabetic group, $^{\triangle}P < 0.05$.

Table 2. Comparison of intestinal flora in each group ($\bar{x} \pm s$, CFU/g).

Group	Case	*Enterobacteria*	*Bacteroides*	*Bifidobacteria*	*Enterococcus*	*Lactobacillus*
Control group	106	7.05 ± 0.41	12.86 ± 1.35	11.56 ± 0.52	6.92 ± 0.66	10.47 ± 0.91
Diabetic group	106	11.63 ± 0.78*	9.51 ± 1.72*	8.04 ± 0.38*	7.05 ± 0.52	7.65 ± 0.48*
Complicated group	106	15.26 ± 1.02$^{\#\star}$	8.03 ± 1.25$^{\#\triangle}$	6.43 ± 0.32$^{\#\triangle}$	6.84 ± 0.39	5.29 ± 0.27$^{\#\triangle}$

Compared with control group, $^{*}P < 0.05$, $^{\#}P < 0.01$; compared with diabetic group, $^{\triangle}P < 0.05$, $^{\star}P < 0.01$.

Table 3. Comparison of levels of inflammatory factors in each group ($\bar{x} \pm s$).

Group	Case	TNF-α (ng/L)	IL-10 (ng/L)	IL-6 (ng/L)	IL-2 (ng/L)	IL-1β (ng/L)	CRP (mg/L)
Control group	106	77.35 ± 4.12	82.36 ± 6.12	27.17 ± 2.78	84.95 ± 3.07	5.76 ± 0.55	4.42 ± 0.26
Diabetic group	106	93.42 ± 6.37$^{\#}$	71.52 ± 7.48$^{\#}$	35.15 ± 6.84*	55.91 ± 2.35$^{\#}$	10.35 ± 0.73$^{\#}$	10.21 ± 0.68$^{\#}$
Complicated group	106	121.45 ± 5.85$^{\#\star}$	57.95 ± 5.23$^{\#\star}$	58.76 ± 4.42$^{\#\star}$	36.77 ± 2.94$^{\#\star}$	19.61 ± 1.15$^{\#\star}$	13.95 ± 0.52$^{\#\triangle}$

TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10; IL-6: interleukin 6; IL-2: interleukin 2; IL-1β: interleukin 1 beta; CRP: C-reactive protein.
Compared with control group, $^{*}P < 0.05$, $^{\#}P < 0.01$; compared with diabetic group, $^{\triangle}P < 0.05$, $^{\star}P < 0.01$.

were risk factors for myocardial ischemia complicated with type 2 diabetes mellitus ($P < 0.05$ or $P < 0.01$), as shown in Table 4.

Discussion

Intestinal flora affected the body's metabolic balance; for example, changes in bacterial structure will lead to intestinal barrier dysfunction of intestinal epithelial cells and impairment of epithelial cell function, which will affect the overall metabolism.[13] In this study, the number of intestinal flora in diabetic patients was different from the normal population. Compared with the diabetic group, the number of *Enterobacteria* in complicated group was increased, while the numbers of *Bacteroides, Bifidobacteria*, and *Lactobacillus* were decreased, indicating that there was more serious intestinal flora imbalance in patients with myocardial ischemia and type 2 diabetes mellitus. *Enterobacteria* is the main source of endotoxin and can secrete NH3 and H2S.[14] After inhibition of *Enterobacteria*, the secreted endotoxins are reduced, which is beneficial to the improvement of symptoms of myocardial ischemia complicated with type 2 diabetes mellitus.[14] This study also found that *Enterobacteria* was

Table 4. Multivariate logistic regression analysis of risk factors for myocardial ischemia complicated with type 2 diabetes mellitus.

Variable	β	SE	Wald χ^2	*P* value	OR (95% CI)
Duration of diabetes	0.416	0.212	4.357	0.027	1.439 (1.054–2.426)
HbA1c	0.017	0.113	5.096	0.018	1.075 (1.003–1.509)
Enterobacteria	0.594	0.226	8.135	0.005	1.761 (1.176–2.852)
TNF-α	1.067	0.317	10.136	0.002	2.674 (1.446–5.133)
IL-6	0.048	0.022	8.795	0.006	1.022 (1.002–1.085)
IL-10	−0.313	0.168	6.124	0.009	0.598 (0.482–0.926)

TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10; IL-6: interleukin 6; SE: standard error; OR: odds ratio; CI: confidence interval.

a risk factor for myocardial ischemia complicated with type 2 diabetes mellitus, which meant that *Enterobacteria* may play a role in the occurrence and development of myocardial ischemia complicated with type 2 diabetes mellitus.

In this study, the duration of diabetes, FBG, 3hPG, and HbA1c in complicated group were higher than those in the diabetic group, but the 2hPG was lower than that in the diabetic group ($P < 0.05$), suggesting that the blood glucose level in patients with myocardial ischemia complicated with type 2 diabetes mellitus was greater. Logistic regression analysis showed that HbA1c and the duration of diabetes were risk factors for myocardial ischemia complicated with type 2 diabetes mellitus ($P < 0.05$).

TNF-α and IL-6 affect the release of gastrointestinal hormones such as motilin and gastrin at the molecular level. IL-10 is associated with the development of diabetes, cardiovascular disease, and other diseases.[15] This study found that compared with control group, the levels of TNF-α, IL-6, IL-1β, and CRP decreased in the diabetic group and complicated group, and the lowest in the complicated group. Conversely, the levels of IL-10 and IL-2 increased in the diabetic group and complicated group, and the highest in the complicated group. Further multivariate logistic regression analysis revealed that TNF-α, IL-6, and IL-10 were the influencing factors of myocardial ischemia complicated with type 2 diabetes mellitus ($P < 0.05$ or $P < 0.01$). Therefore, it can be speculated that intestinal flora and inflammatory factors play a role in the occurrence and development of myocardial ischemia complicated with type 2 diabetes mellitus, but the specific mechanisms and causality should be further verified by animal experiments.

In summary, *Enterobacteria*, TNF-α, IL-6, and IL-10 may play an important role in the development of myocardial ischemia in type 2 diabetes mellitus.

Declaration of conflicting interests

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