European Review for Medical and Pharmacological Sciences 2020; 24: 10162-10168

Correlations between inflammatory response, oxidative stress, intestinal pathological damage and intestinal flora variation in rats with type 2 diabetes mellitus

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Abstract. – OBJECTIVE: To explore the correlations between inflammatory response, oxidative stress, intestinal pathological damage, and intestinal flora variation in rats with type 2 diabetes mellitus (T2DM).

MATERIALS AND METHODS: A total of 80 specific pathogen-free (SPF) male Sprague-Dawley (SD) rats purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijiing, China) were randomly divided into two groups, namely T2DM group (n=40) and normal group (n=40). Then, the contents of inflammatory factors [high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-α (TNF-α)] and oxidative stress indicators [malondialdehyde (MDA) and superoxide dismutase (SOD)] were detected. Meanwhile, the distributions of intestinal florae Bifidobacteria, Escherichia coli, Lactobacilli and Enterococcocci and the variation of endotoxin were compared between the two groups. Besides, colon specimens were pathologically examined to observe the occurrence of chronic inflammation variation, intestinal mucosal erosion, intestinal mucosal wall thickening and intestinal mucosal fibrosis. Next, the influences of the glycosylated hemoglobin on levels of hs-CRP, MDA and endotoxin and colonization ability of intestinal florae were analyzed. Additionally, univariate and multivariate analyses were performed for underlying the relations of the pathogenesis of T2DM in rats with their inflammatory response, antioxidant capacity, endotoxin level and intestinal flora colonization capacity.

RESULTS: The levels of hs-CRP and TNF-α were significantly higher in T2DM group than those in normal group ($p<0.05$). T2DM group exhibited an overtly elevated MDA level ($p<0.05$), and a clearly lowered SOD level ($p<0.05$) in comparison with normal group. As to intestinal flora-related indicators, the levels of endotoxin, Escherichia coli and Enterococcocci were evidently higher in T2DM group than those in the normal group ($p<0.05$), while the levels of Bifidobacteria and Lactobacilli in T2DM group were remarkably lowered ($p<0.05$). Pathological lesions, including chronic inflammation variation, mucosal erosion, mucosal wall thickening and mucosal fibrosis in intestinal mucosal tissues, were worse in T2DM group than those in the normal group ($p<0.05$). In T2DM rats, the level of glycosylated hemoglobin was positively correlated with changes in the levels of hs-CRP, MDA and endotoxin ($p<0.05$), and negatively associated with changes in colonization ability of intestinal florae ($p<0.05$). Aggravated inflammatory response, decreased antioxidant capacity, increased endotoxin level and weakened colonization ability of intestinal florae were independent risk factors for T2DM in rats.

CONCLUSIONS: Rats with T2DM have significantly aggravated inflammatory response, weakened antioxidant capacity, imbalanced intestinal florae and markedly pathological changes of intestinal mucosa.

Key Words:
MiRNA-106, Pediatric osteosarcoma, PI3K/AKT signaling pathway.

Introduction

With the changes in the living habits and dietary habits of people in China in recent years, type 2 diabetes mellitus (T2DM) has a markedly elevated incidence rate, which has become one of the chronic non-communicable diseases with the highest incidence rate in China[1]. T2DM has a long course, poor prognosis and many complications, seriously affecting the life quality and health[2]. Insulin resistance, which is regarded as an initial factor and a main mechanism of T2DM, participates in the entire development and pro-

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gression of T2DM. It mainly leads to an increase in the glycosylated hemoglobin level and glucose metabolism disorders[3].

In the case of T2DM, the biological function of insulin impairs, and compensatory secretion of insulin will increase[4]. Moreover, impaired blood glucose metabolism leads to continuous chronic inflammatory response and reduced antioxidant capacity in the body, further promoting the progression of insulin resistance[5]. In addition, long-term insulin resistance will further damage vascular endothelial cell function, blood lipid metabolism and gastrointestinal function[6]. However, T2DM-induced damages in gastrointestinal functions, especially pathological changes of the gastrointestinal tract, is rarely studied. In this study, therefore, T2DM model in rats was established, and the inflammation response, oxidative stress, intestinal pathological damage and intestinal flora variation in T2DM rats were analyzed.

Materials and Methods

General Data

A total of 80 specific pathogen-free (SPF) male Sprague-Dawley (SD) rats (qualification number: SCXK (Jing) 2018-0001) weighing (200±20) g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). They were fed in the SPF Animal Experiment Center of our hospital, and divided into T2DM group (n=40) and normal group (n=40) using a random number table. All laboratory rats were adaptively fed for 1 week under a 12 h/12 h light cycle and they had free accesses to water and food. The laboratory room temperature was controlled at about 24°C, and the humidity was controlled at 40-50%. This study was approved by the Animal Ethics Committee of Beijing Chao-Yang Hospital Animal Center.

Modeling of T2DM and Normal Rats

Rats in T2DM group were adaptively fed for 1 week and then fed with high-fat and high-sugar diet containing 67.5% rat maintenance feed, 10% lard, 20% sucrose and 2.5% egg yolk powder for consecutive 4 weeks. After overnight fast, they were intraperitoneally injected with 30 mg/kg STZ solution prepared in sodium citrate buffer (pH 4.4) every day. 7 days later, blood was drawn from caudal vein to measure fasting blood glucose in rats after they were deprived of food and water for 12 h. Blood glucose ≥7.8 mmol/L suggested successful modeling. The remaining 40 rats in normal group were fed normally for consecutive 6 weeks (1 week of adaptive feeding followed by 5 weeks of feeding with normal diet). After that, they were deprived of food and water for 12 h, and then, blood was drawn from caudal vein to measure fasting blood glucose in rats. Blood glucose <11.1 mmol/L indicated normal. Subsequently, fasting blood was collected in the morning from the tain vein for examination of biochemical indicators. They were sacrificed by cervical dislocation to collect colon specimens for pathological examination.

Grouping and Index Observation

The contents of inflammatory response indexes [high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-α (TNF-α)] and oxidative stress indicators [malondialdehyde (MDA) and superoxide dismutase (SOD)] were detected. The distribution of intestinal florae *Bifidobacteria, Escherichia coli, Lactobacilli and Enterococcocci*, and the variation of endotoxin were compared between the two groups. Besides, colon specimens were pathologically examined for the occurrence of chronic inflammation variation, intestinal mucosal erosion, intestinal mucosal wall thickening, and intestinal mucosal fibrosis. Next, the influences of glycosylated hemoglobin on levels of hs-CRP, MDA and endotoxin and colonization ability of intestinal florae were analyzed. Additionally, univariate and multivariate analyses were performed for underlying the pathological influences of T2DM on inflammatory response, antioxidant capacity, endotoxin level and intestinal flora colonization capacity.

Evaluation Criteria

Evaluation criteria for inflammatory response: normal hs-CRP: ≤10 mg/L, and normal TNF-α: 5-100 ng/L; oxidative capacity: normal MDA: 3.52-4.78 nmol/mL, normal SOD: 242-620 μU/mL, normal endotoxin level: <0.1 EU/mL (azo color method), and normal glycosylated hemoglobin: 4-6% (HbAlc, liquid chromatography). Detection of intestinal flora: *Bifidobacterium, Escherichia coli, Lactobacillus* and *Enterococcus* levels were mainly measured. The colonization ability of intestinal florae was assessed based on the ratio of Bifidobacterium to *Escherichia coli* (B/E), with B/E >1 for normal, and B/E <1 for intestinal flora imbalance.

Table I. Comparisons of inflammatory response, oxidative stress and intestinal flora distribution between two groups ($\bar{x} \pm s$).

	Hs-CRP (mg/L)	TNF-α (ng/L)	MDA (nmol/mL)	SOD (μU/L)	Endotoxin (EU/mL)
T2DM group	14.5 ± 0.9	161.4 ± 9.3	8.9 ± 1.8	187.9 ± 18.1	0.99 ± 0.1
Normal group	5.0 ± 0.2	56.9 ± 6.6	4.8 ± 1.5	332.7 ± 27.6	0.06 ± 0.1
t	65.169	57.955	11.067	27.747	41.591
p	0.000	0.000	0.000	0.000	0.000

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was employed for statistical analysis. Measurement data, such as indicators of inflammatory response, oxidative stress and intestinal flora, glycosylated hemoglobin content, and intestinal flora colonization capability were expressed as mean ± standard deviation (χ±s). The *t*-test was used to compare differences between two groups. Enumeration data were compared using χ^2 test. Univariate analysis and multivariate Logistic regression analysis were conducted to assess risk factors in T2DM. Meanwhile, Pearson method was adopted for correlation analysis. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of Inflammatory Response, Oxidative Stress and Intestinal Flora Distribution Between Two Groups

Compared with those in the normal group, the levels of hs-CRP, TNF-α, MDA and endotoxin dramatically increased, while the level of SOD overtly declined in T2DM group ($p<0.05$) (Table I).

Comparison of Intestinal Flora Distribution Between Two Groups

T2DM group had lower *Bifidobacterium* and *Lactobacillus* levels and higher *Escherichia coli* and *Enterococcus* levels in comparison with normal group ($p<0.05$) (Table II).

Comparisons of Pathological Characteristics of Intestinal Mucosal Tissues Between Two Groups

Chronic inflammation variation, mucosal erosion, mucosal wall thickening and mucosal fibrosis in intestinal mucosal tissues were worse in T2DM group than those in the normal group ($p<0.05$) (Table III).

Correlations of Glycosylated Hemoglobin Level with hs-CRP, MDA and Endotoxin Content and Intestinal Flora Colonization Ability

Correlation analysis results revealed that the level of glycosylated hemoglobin in T2DM rats

Table II. Comparison of intestinal flora distribution between two groups (LgN/g, $\bar{x} \pm s$).

	Bifidobacterium	*Escherichia coli*	*Lactobacillus*	*Enterococcus*
T2DM group	7.3 ± 0.6	9.1 ± 0.8	6.1 ± 0.7	7.3 ± 0.6
Normal group	9.6± 0.8	8.2 ± 0.6	7.9 ± 0.8	6.3 ± 0.5
t	14.546	5.692	10.709	8.098
p	0.000	0.000	0.000	0.000

Table III. Comparisons of pathological characteristics of intestinal mucosa tissues between two groups [n (%)].

	Chronic inflammation variation	Mucosal erosion	Mucosal wall thickening	Mucosal fibrosis
T2DM group	32	31	30	12
Normal group	1	2	1	3
χ^2	46.422	40.438	41.290	5.251
p	0.000	0.00	0.000	0.022

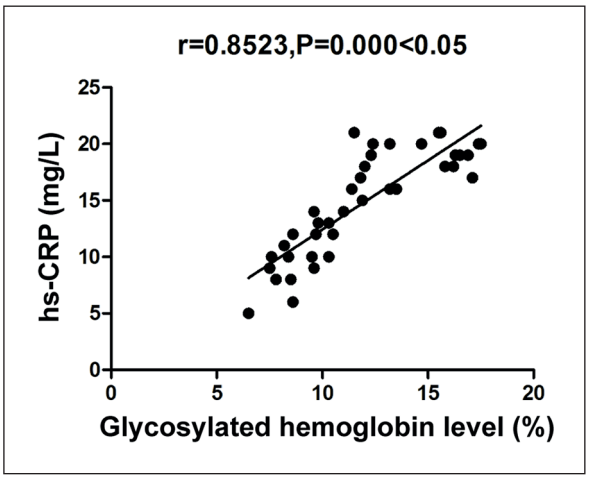

Figure 1. Correlation between glycosylated hemoglobin level and hs-CRP level.

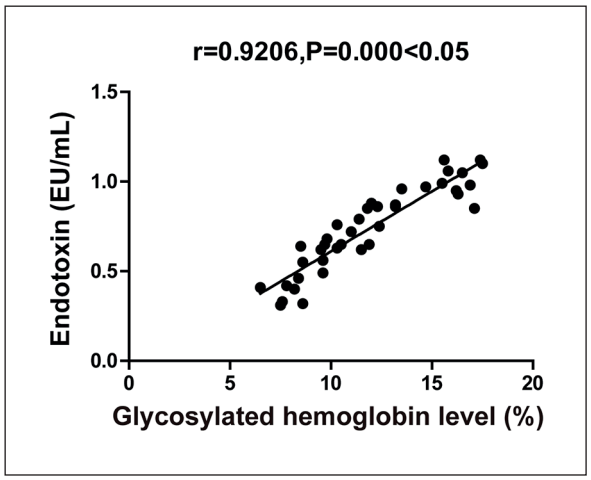

Figure 3. Relation between glycosylated hemoglobin level and endotoxin level.

had a positive association with the changes of hs-CRP, MDA and endotoxin levels (p<0.05) (Figure 1, 2 and 3) and a negative relation with the colonization ability of intestinal florae (p<0.05) (Figure 4, Table IV).

Univariate Analysis of Correlations of the Development of T2DM in Rats with Inflammatory Response, Antioxidant Capacity, Endotoxin Level and Colonization Capacity of Intestinal Florae of Rats

Based on univariate analysis, it was uncovered that inflammatory response, antioxidant capacity, endotoxin level and colonization capacity of intestinal florae in rats were relevant risk factors for T2DM in rats (Table V).

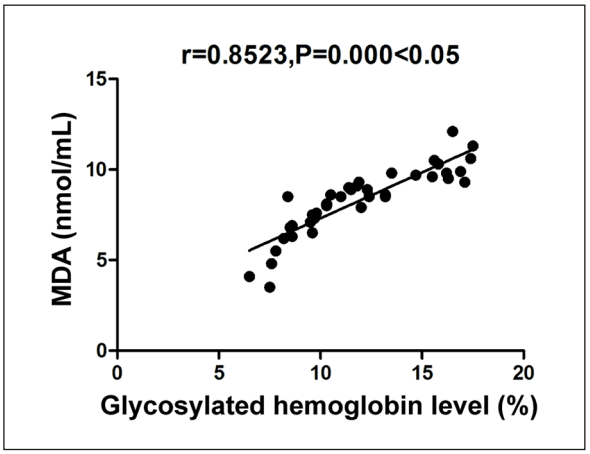

Figure 2. Association between glycosylated hemoglobin level and MDA level.

Multivariate Logistic Regression Analysis of Correlations of the Development of T2DM in Rats with Inflammatory Response, Antioxidant Capacity, Endotoxin Level and Colonization Capacity of Intestinal Florae of Rats

Multivariate logistic regression analysis results showed that aggravated inflammatory response, weakened antioxidant capacity, raised endotoxin level and reduced colonization capacity of intestinal florae were independent risk factors for T2DM in rats (Table VI).

Discussion

T2DM, the most common chronic endocrine and metabolism-related disease, is mainly char-

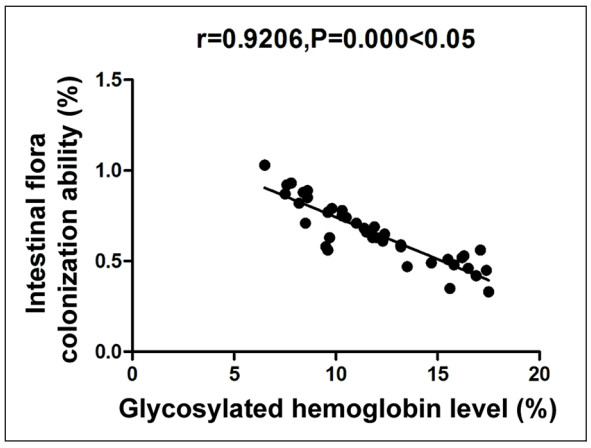

Figure 4. Relationship between glycosylated hemoglobin level and intestinal flora colonization ability.

Table IV. Correlations of glycosylated hemoglobin level with hs-CRP, MDA and endotoxin content and intestinal flora colonization ability.

	r	*p*
Hs-CRP level	0.8523	0.000
MDA level	0.8823	0.000
Endotoxin level	0.9206	0.000
Intestinal flora colonization ability	-0.8930	0.000

acterized by elevated blood glucose, glycosylated hemoglobin and insulin levels, and insulin resistance[7]. Due to its long course and many complications, it has a great negative impact on the life quality and even the living conditions of patients. T2DM brings certain economic burden on the countries, patient families and individuals[8]. Besides, the incidence of T2DM tends to show a younger trend. Numerous studies have demonstrated that insulin resistance is a key central link in the pathogenesis of T2DM. In the case of insulin resistance in the body[9], the inflammatory response of the body is evidently enhanced, while the antioxidant capacity decreases, thereby further aggravating insulin resistance in the body[10]. Moreover, the changes in the intestinal florae of T2DM patients are closely correlated with the development and progression of T2DM[11]. Gribble et al[12] showed that most T2DM patients suffer severe intestinal flora imbalance that is mainly manifested as reduced *Bifidobacterium* and *Lactobacillus* contents and elevated *Enterococcus* and *Faecoccus* contents[13]. Many studies have confirmed that T2DM is associated with inflammatory response, antioxidant capacity, and intestinal flora variation in the body, but there are few studies on T2DM and intestinal pathological changes.

In this study, inflammatory response, oxidative stress, and intestinal flora distribution were compared between two groups. It was discovered that the levels of hs-CRP (an inflammatory response indicator), TNF-α (an inflammatory response index), MDA (an oxidative stress capacity indicator) and endotoxin (an intestinal flora-related index) dramatically increased, while the level of SOD (an oxidative stress capacity indicator) overtly declined in T2DM group. It is suggested that T2DM resulted in chronic inflammatory response, impaired antioxidant capacity, and a raised level of intestinal endotoxin, which may be related to the imbalance of conventional florae and an increase in Gram-negative bacteria. In addition, comparison of intestinal flora distribution between the two groups revealed that T2DM group had lowered *Bifidobacterium* and *Lactobacillus* levels and increased *E.coli* and *Enterococcus* levels than those in normal group. It is suggested that T2DM leads to increases in Gram-positive

Table V. Univariate analysis of correlations of the development of T2DM in rats with inflammatory response, antioxidant capacity, endotoxin level and colonization capacity of intestinal florae of rats.

Item		T2DM	Normal	χ^2	*p*
Levels of inflammatory factors	Increase	26	25	0.054	0.816
	Normal	14	15		
Antioxidant capacity	Decrease	27	14	8.455	0.004
	Normal	13	26		
Endotoxin level	Increase	33	21	8.205	0.004
	Normal	7	19		
Intestinal flora colonization ability	Normal	8	25	14.907	0.000
	Decrease	32	15		

Table VI. Multivariate logistic regression analysis of correlations of the development of T2DM in rats with inflammatory response, antioxidant capacity, endotoxin level and colonization capacity of intestinal florae of rats.

Item	β	SE	W	OR	*p*	95% CI
Levels of inflammatory factors	0.145	0.390	0.139	0.211	0.709	0.539-2.478
Antioxidant capacity	-1.605	0.382	17.717	4.972	0.000	2.354-6.490
Endotoxin level	0.328	0.371	0.982	0.281	0.459	0.619-2.624
Intestinal flora colonization ability	-1.049	0.440	5.646	2.853	0.017	1.201-6.773

bacteria like *Bifidobacterium* and *Lactobacillus*, Gram-negative bacilli, such as *Escherichia coli* and *Enterococcocci*. Furthermore, the pathological characteristics of intestinal mucosal tissues were compared between two groups. The ratio of cases with chronic inflammation variation, mucosal erosion, mucosal wall thickening and mucosal fibrosis in intestinal mucosal tissues was larger in T2DM group than that in normal group. We found that the level of glycosylated hemoglobin in T2DM rats had a positive association with the changes of hs-CRP, MDA and endotoxin levels and a negative relation to the colonization ability of intestinal florae. Lastly, univariate and multivariate analysis were employed for the correlations of the development of T2DM in rats with inflammatory response, antioxidant capacity, endotoxin level and colonization capacity of intestinal florae of rats. It was found that aggravated inflammatory response, weakened antioxidant capacity, raised endotoxin level and reduced colonization capacity of intestinal florae were independent risk factors for T2DM in rats.

T2DM-induced impaired islet function and insulin resistance lead to evident glucose metabolism disorders, including fasting and postprandial blood glucose elevation, and elevated glycosylated hemoglobin level[14]. In addition, insulin resistance in the body will cause increased inflammatory response, decreased antioxidant capacity and protein kinase C activity, and elevated end-glycosylation products in the body[15]. It activates the nuclear factor κB system and promotes the body to keep hyperglycemic memory, thus damaging the vascular endothelium of the body. Eventually, vascular endothelial cells are damaged[16]. Furthermore, studies have shown that there is intestinal microbial flora imbalance due to T2DM-induced insulin resistance[17], namely lowered levels of *Bifidobacteria* and *Lactobacillus* and raised levels of *Enterococcocci* and *Escherichia coli*. Such an intestinal flora variation is more evident in the body with remarkably elevated content of glycosylated hemoglobin[18]. In addition to intestinal flora variation[19], T2DM will further give rise to changes in the intestinal pathological structure, which may be associated with damaged intestinal mucosa and increased intestinal permeability. Short-chain fatty acid and bile acid metabolism alteration due to stimuli like long-term chronic inflammation and endotoxin level increase may explain the pathological conditions[20].

Conclusions

Prominently aggravated inflammatory response, weakened antioxidant capacity, imbalanced intestinal florae and remarkable pathological injury of intestinal mucosa are detected in rats with T2DM.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This study was supported by National Natural Science Foundation of China (No. 81670519) and Beijing Hospitals Authority Youth Programme (No. QML20190302).

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