Diversity of Intestinal Flora in Elderly Patients with Type 2 Diabetes Mellitus with Early Nephropathy

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Abstract: *Objective:* To investigate the intestinal flora in elderly patients with type 2 diabetes mellitus with early nephropathy. *Methods:* 43 elderly patients with type 2 diabetes mellitus with early nephropathy (diabetic nephropathy group) and 51 elderly patients with type 2 diabetes mellitus (type 2 diabetes mellitus group) admitted to our hospital from January 2021 to October 2022 were retrospectively analyzed, with 39 healthy people who underwent a physical examination in our hospital during the same period as the control group. The fecal specimens of the three groups were collected, and the 16S rDNAs of bacteria in the fecal samples were extracted, amplified, and sequenced for intestinal flora operational taxonomic unit (OTU) classification and Alpha diversity analysis. *Results:* (1) Intestinal flora OTUs: there were 545 intestinal flora OTUs unique to the control group, 424 intestinal flora OTUs unique to diabetic nephropathy, and 321 intestinal flora OTUs unique to the type 2 diabetes group. There were 403 intestinal flora OTUs common to the control group and diabetic nephropathy group, 256 intestinal flora OTUs common to the control group and type 2 diabetes group, and 298 intestinal flora OTUs common to the type 2 diabetes group and diabetic nephropathy group. 235 intestinal flora OTUs were common to all 3 groups of subjects. (2) Alpha diversity: The statistical analysis indicated that there was a statistically significant difference ($P < 0.05$) in the Alpha diversity of intestinal flora, as assessed by the Ace index and Simpson's index, among the three subject groups. However, no statistical significance ($P > 0.05$) was observed when comparing the Chao 1 index and Shannon index. Further observation of the Ace index and Simpson index in the three groups revealed that both the diabetic nephropathy group and the type 2 diabetes mellitus group had lower values than the control group. *Conclusion:* The diversity of intestinal flora decreases in elderly patients with type 2 diabetes mellitus with early nephropathy.

Keywords: Elderly type 2 diabetes mellitus; Nephropathy; Intestinal flora; Diversity

1. Introduction

According to the National Bureau of Statistics, in the seventh national census, the number of people aged 60 years and above has reached 260 million, and China has already stepped into an aging society [1]. Among these 260 million elderly people, about 78 million people suffer from diabetes, and about 95% of these diabetic patients have type 2 diabetes [2]. This number will continue to increase as the population ages. Diabetic

nephropathy is a common clinical complication of diabetes mellitus, and data show that 30–40% of diabetic patients will develop diabetic nephropathy [3], and early diagnosis and treatment are important for slowing down the progression of the disease [4].

In recent years, several studies have shown that intestinal flora is one of the important factors affecting the occurrence and development of early diabetic nephropathy, and some studies have shown that disruption of intestinal flora affects insulin sensitivity and plays a role in the pathogenesis of type 2 diabetes mellitus [5]. Intestinal flora plays an important role in human health. Dysregulation of intestinal flora can bring about the occurrence of various diseases, and relevant studies have shown that with further aggravation of the disease in patients with early diabetic nephropathy, the diversity of intestinal flora in patients will also decrease [6]. Therefore, it is necessary to study the changes in the intestinal flora of elderly patients with type 2 diabetes mellitus with early nephropathy. The progression of the disease can be determined based on the changes in the diversity of intestinal flora, and it is helpful for the prevention and treatment of type 2 diabetes mellitus. In this paper, we analyzed the changes in the diversity of intestinal flora in elderly type 2 diabetic patients to provide a theoretical basis for related clinical prevention and treatment.

2. Information and methods

2.1. General information

43 patients with type 2 diabetes mellitus with early nephropathy (diabetic nephropathy group) and 51 patients with type 2 diabetes mellitus (type 2 diabetes mellitus group) admitted from January 2021 to October 2022 were selected as the study subjects; 39 healthy patients who underwent physical examination in our hospital during the same period were selected as the control group.

Inclusion criteria: (1) over 60 years old, (2) diagnosed with type 2 diabetes mellitus and nephropathy, with a urinary microalbumin/creatinine ratio between 30 mg/g and 299 mg/g; (3) the control group was free of diabetes mellitus, hypertension, and dyslipidemia, and had not taken antibiotics or micro-ecological agents such as probiotics in the 2 weeks before sampling.

Exclusion criteria: (1) patients with type 1 diabetes mellitus, gestational diabetes mellitus, or other special types of diabetes mellitus; (2) combination of primary and other secondary renal diseases, such as hypertensive nephropathy, glomerulonephritis, etc.; (3) diabetes mellitus-type complications, such as hyperglycemic and hyperosmolar states; (4) no history of surgery in the last 6 months; (5) had taken antibiotics or microecological agents such as probiotics in the 2 weeks prior to sampling.

2.2. Methods

1 g of stool sample from the patient was taken and placed in a sterile container containing preservation solution. 0.1g of the sample was taken and put into a sterile centrifuge tube, and sterile saline or buffer was added to dilute the feces. The sample was then ground into a homogeneous suspension using a sterile grinder or homogenizer, and then centrifuged. The 16S rDNA of the bacteria in the fecal sample was extracted, amplified, and then sequenced by a high-throughput sequencing technology for the division of intestinal flora OTUs. The Ace index, Chao 1 index, Shannon index, and Simpson's index were selected was carried out to compare the richness and diversity of flora between different samples.

2.3. Statistical analysis

The data were analyzed using SPSS 20.0. Normality tests were conducted for the measurement data. Measurement data were expressed as (mean ± standard deviation), and comparisons were made using split-

parameter tests. *P* < 0.05 indicated a statistically significant difference.

3. Result

3.1. Intestinal flora OTUs in the 3 groups of subjects

There were 545 intestinal flora OTUs unique to the control group, 424 intestinal flora OTUs unique to the diabetic nephropathy group, and 321 intestinal flora OTUs unique to the type 2 diabetes group. There were 403 intestinal flora OTUs common to the control and diabetic nephropathy group, 256 intestinal flora OTUs common to the control and type 2 diabetes group, and 298 intestinal flora OTUs common to the type 2 diabetes group and diabetic nephropathy group. Lastly, there were 235 intestinal flora OTUs common to the 3 groups of subjects.

3.2. Alpha diversity of intestinal flora in 3 groups of subjects

there was a statistically significant difference (*P* < 0.05) in the Alpha diversity of intestinal flora, as assessed by the Ace index and Simpson's index, among the three subject groups. However, no statistical significance (*P* > 0.05) was observed when comparing the Chao 1 index and Shannon index. Further observation of the Ace index and Simpson index in the three groups revealed that both the diabetic nephropathy group and the type 2 diabetes mellitus group had lower values than the control group. The details are shown in **Table 1**.

Table 1. Alpha diversity of intestinal flora in 3 groups of subjects

Group	Ace index	Chao 1 index	Shannon index	Simpson's index
Diabetic nephropathy group (*n* = 43)	926.58 ± 265.21	911.28 ± 282.35	6.25 ± 1.48	0.96 ± 0.25
Type 2 diabetes group (*n* = 51)	845.28 ± 275.23	925.47 ± 278.36	6.28 ± 1.77	0.95 ± 0.29
Control group (*n* = 39)	998.15 ± 282.65	968.28 ± 288.14	6.68 ± 1.88	1.15 ± 0.25
F	3.477	0.449	0.804	7.450
P	*P* < 0.05	*P* > 0.05	*P* > 0.05	*P* < 0.05

4. Discussion

Type 2 diabetic nephropathy is a relatively common complication of diabetes. The complications tend to be more severe in elderly patients, and they have higher risk factors. This will cause multiple hazards to their health and quality of life.

Intestinal flora plays an important role in the human body and is of great significance to the maintenance of human health and disease prevention. Intestinal flora can not only help digest and absorb nutrients such as proteins, fats, and carbohydrates, but also stimulate the development and activation of immune cells, enhance the body's immunity, and prevent infections and diseases. Besides, it also effectively maintains the intestinal barrier function, promotes the growth and repair of intestinal cells, enhances the integrity of the intestinal barrier, and prevents the entry of bacteria, viruses, and toxins into the body [7].

Studies have shown that intestinal flora plays a positive role in the treatment of several diseases. Jiang *et al.* showed that intestinal flora plays a role in glaucoma, diabetic retinopathy, and uveitis [8]. Peng *et al.* showed that intestinal flora helps to reduce adverse reactions in tumor treatment and aids the inhibition of tumor progression, thus optimizing the clinical treatment of tumors and improving the survival of patients with tumors [9]. In addition to this, there have also been many studies showing that intestinal flora plays a role in depression, [10]

Alzheimer's disease [11], and other diseases.

The results of OTU classification in this study suggest that there are some similarities as well as differences in the intestinal flora of patients with diabetic nephropathy, type 2 diabetes mellitus, and healthy patients [12]. Besides, the Alpha diversity results indicated that the decrease in the diversity of intestinal flora may be related to the onset and progression of the disease. These results are consistent with a study by Zheng *et al.* that showed that the exacerbation of type 2 diabetic nephropathy patients and the short-term progression of albuminuria were related to the decrease in the diversity of intestinal flora in patients [13].

Intestinal flora is closely related to the occurrence and development of early diabetic nephropathy in the elderly, so it is of great significance to explore the regulation of intestinal flora in clinical treatment. Some studies have shown that probiotic supplements such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* can promote intestinal peristalsis, effectively reduce the content of enterogenic urotoxins in the blood of patients, and improve the microinflammatory state of the patient's body, effectively preventing the deterioration of renal function [14]. Traditional Chinese medicine probiotics can also be given as a supplement [15].

5. Conclusion

In summary, the diversity of intestinal flora in elderly patients with early-stage nephropathy and type 2 diabetes mellitus is reduced compared to healthy individuals and those with uncomplicated diabetes. The dysregulation of intestinal flora may be associated with the development and progression of early-stage nephropathy in type 2 diabetes mellitus. Future research should focus on methods to regulate intestinal flora in this context.

Disclosure statement

The authors declare no conflicts of interest.

References

[1] Zhou Q, Yang S, 2023, Analysis of The Health Status of the Elderly Population in China Under the Background of Actively Coping with The Aging Population Policy: Based on the Comparative Analysis of the Sixth and Seventh National Population Censuses. Population and Health, 2023(07): 49–53.

[2] Yang X, Yuan L, Ye Z, et al., 2022, Interpretation of the Comprehensive Health Assessment in the Chinese Clinical Guidelines for the Prevention and Control of Type 2 Diabetes Mellitus in the Elderly (2022 Edition). Practical Geriatrics, 36(11): 1180–1184.

[3] Wang F, Wang W, 2022, Interpretation of the Chinese Clinical Guidelines for the Prevention and Control of Type 2 Diabetes Mellitus in the Elderly (2022 Edition). Journal of Hebei Medical University, 43(12): 1365–1370.

[4] Qiu X, 2022, The Significance of Urine Protein Test in the Diagnosis of Early Diabetic Nephropathy. Primary Medical Forum, 26(17): 127–129.

[5] Dabke K, Hendrick G, Devkota S, 2019, The Gut Microbiome and Metabolic Syndrome. J Clin Invest, 129(10): 4050–4057.

[6] Mejers B, Jouret F, Evenepoel P, 2018, Linking Gut Microbiota to Cardiovascular Disease and Hypertension: Lessons From Chronic Kidney Disease. Pharmacol Res. 133: 101–107.

[7] Sun H, 2023, Analysis of Intestinal Flora and Inflammatory Factors and T-cell Subsets in Pregnant Women with Gestational Diabetes Mellitus. Journal of Practical Examiner, 15(01): 22–25.

[8]   Jiang J, Lu P, 2023, Progress in the Study of the Role of Intestinal Flora in Ocular Diseases. International Journal of Ophthalmology, 23(07): 1153–1157.

[9]   Peng C, Chen J, Chai K, et al., 2023, Research Progress of Intestinal Flora in Tumor Application. Journal of Practical Oncology, 38(03): 290–293.

[10] Wu Z, Wang Q, Qin X, et al., 2019, Research Progress of Intestinal Flora and its Metabolites in the Treatment of Depression with Traditional Chinese Medicine. Chinese Herbal Medicine, 54(14): 4713–4721.

[11] Lei X, Dong L, Li J, et al., 2019, Relationship Between Intestinal Flora and Alzheimer's Disease at Different Ages. Chemistry of Life, 43(06): 872–880.

[12] Chu H, Li L, 2021, Diversity of Intestinal Flora in Elderly Patients with Type 2 Diabetes Mellitus with Early Nephropathy. Chinese Journal of Microecology, 33(08): 916–919.

[13] Zheng X, Cao Y, 2023, Effect of Intestinal Flora on Renal Function Impairment and Short-Term Progression of Albuminuria in Patients with Type 2 Diabetic Nephropathy. Chinese Journal of Microecology, 35(04): 426–430.

[14] Tang W, Liu N, Fan B, 2020, Effect of Probiotic Supplementation on the Risk of Disease Progression in Elderly Patients with Diabetes in Yukon. Chinese Journal of Microecology, 2020(05): 570–574.

[15] Zou T, Peng T, 2022, Research Progress of Exploring Traditional Chinese Medicine Against Diabetic Nephropathy Based on Intestinal Flora. Journal of Northwest University for Nationalities (Natural Science Edition), 43(03): 58–62.