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Alaa Mohamed Rabea[1], Mohamed Elsheikh[1], Radwa Ahmed Rabea Abdel-Latif[2], May S. Soliman[3], Amani El-Kholy[3], Ahmed Amin Ibrahim[1], Khaled Elsayed Elhadidy[1], Mahmoud Hassan[1]

[1]Department of Internal Medicine, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

[2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

[3]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

Gut Dysbiosis and Diabetic Nephropathy Progression in Patients with Type 2 Diabetes: A Case-Control Study

ABSTRACT

Objective: Diabetic nephropathy (DN) is a leading cause of end-stage renal disease (ESRD). There is growing evidence that intestinal dysbiosis is associated with various disorders. This study aimed to investigate the associations between the human gut microbiome and the development of DN.

Materials and methods: This case-control study was carried out on 75 subjects, both sexes, divided into 3 equal groups (n = 25): Group I consisted of healthy participants; Group II included individuals who had type 2 diabetes (T2D) but no nephropathy; and Group III was identified as having DN based on a urine albumin creatinine ratio of 30 mg/g or above. DNA extraction using PCR amplification and 16S rRNA gene sequencing were used to analyze the fecal microbiota.

Results: Twenty-five healthy controls (12 women and 13 men) had a mean age of 45 ± 8.77 years. Of the 25 patients with T2D, 11 were females, 14 were males, and the mean age was 45 ± 6.68 years. The average age of the 25 DN patients (10 females and 15 males) was 45 ± 6.68 years. Urinary albumin creatinine ratios were found to positively correlate with *Escherichia-Shigella* (r = 0.88, p-value < 0.002) and *Alistipes* (r = 0.91, p < 0.0001), respectively. A negative association (r = –0.77, p-value < 0.0001) was detected between the [*Ruminococcus*] torques group and the estimated glomerular filtration rate (eGFR).

Conclusions: Unbalanced gut microbiota significantly correlates with clinical markers of renal function, cholesterol, blood albumin, and urine albumin creatinine ratio. (Clin Diabetol 2024; 13, 4: 193–199)

Keywords: gut microbiota, dysbiosis, diabetic nephropathy, type 2 diabetes

Address for correspondence:
Mohamed Elsheikh
Department of Internal Medicine, Faculty of Medicine, Beni-Suef University, Beni- Suef 18116, Egypt
E-mail: MohamedElsheikh2022@hotmail.com
Phone: 00201559382002

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Introduction

The gut microbiota of humans has a complex and dynamic relationship with the host, but it also works synergistically to benefit all parties involved. This explains why the bacterial community in the human gut is so diverse and dynamic, and why the environment there is highly complex [1]. Dysbiosis encourages the growth of bacteria that create uremic toxins, such as trimethylamine-N-oxide (TMAO), indoxyl sulfate (IS), p-cresyl sulfate (p-CS), and indole-3-acetic acid (IAA), which accumulate in chronic kidney disease (CKD) [2].

Moreover, inflammation, immunological imbalance, and the translocation of lipopolysaccharides (LPS) are caused by the breakdown of epithelial tight junctions caused by dysbiosis [3, 4].

Serious consequences, including long-term mortality from cardiovascular disease (CVD), comorbidities like protein-energy wasting, and the advancement of CKD towards end-stage renal disease (ESRD), are associated with these alterations in the uremic milieu [5]. In addition to uremia, dysbiosis is caused by the accumulation of metabolites such as uric acid, inadequate fiber intake, and multiple treatment regimens that alter the biochemical environment in the uremic intestines [6]. Several nutritionally based therapeutic strategies have been proposed to alleviate uremic dysbiosis, such as low-protein diets, probiotics, prebiotics, synbiotics, and bioactive compounds [7].

Alzheimer's, CKD, diabetes, obesity, cardiovascular disease, and dysbiosis are all linked to these conditions [8]. Diabetic nephropathy (DN), the leading cause of ESRD in many parts of the world, can strike about 35% of all people with diabetes [9, 10]. Chronic low-level inflammation has recently been linked to additional pathogen-host interaction pathways, such as gut microbiota (GM) and the innate immune system. Diabetes and its consequences, including DN, are already known to be significantly influenced by chronic low-grade inflammation [11].

The study aims to describe and analyze the clinical features and the microbiome characteristics of patients with DN compared to healthy controls.

Materials and methods

Study design

Seventy-five participants of both sexes participated in this case-control study between May 2021 and November 2022. Every participant provided written consent after being fully briefed. Patients with hepatitis C and hepatitis B viruses, chronic illnesses, acute intercurrent infections, and/or those using antibiotics, probiotics, or immunosuppressive medications were excluded.

Study population/study participants

Subjects were divided into 3 equal groups: Group I (control group) — healthy volunteers with no previous medical history of diabetes or other illnesses; Group II — patients with type 2 diabetes (T2D) without nephropathy; and Group III — diabetic nephropathy, which was defined by a urinary albumin creatinine ratio ≥ 30 mg/g.

Ethical approval

We conducted our study after clearance from the Ethics Committee (approval number: FMBSUREC/ /06042021/Elsheikh).

Data collection/variables

Each participant underwent the following procedures: taking a complete medical history, a thorough physical examination, standard investigations (serum creatinine, BUN, fasting and postprandial blood glucose, HbA1c, urine albumin creatinine ratio, lipid profile, immune profile, virology (HBsAg, HCV Ab, anti-HIV), fundus examination, estimated glomerular filtration rate, analysis of the fecal microbiota using 16S rRNA gene sequencing, and DNA extraction by PCR amplification (next generation sequencing). Regarding the collection of fecal samples, all participants provided at least 1 g of fresh, solid intestinal feces, which were then frozen at -80°C in sterile tubes for future research. The QIAamp Fast DNA Stool Mini Kit from Qiagen was used to obtain DNA from stool specimens according to the manufacturer's supplied directions (Cat # 51604, Qiagen). To prepare the 16S Metagenomic Sequencing Library, Part # 15044223 Rev. A: Illumina, San Diego, CA, USA, the Illumina 16S Metagenomic Sequencing Protocol was followed. Using the MiSeq Reagent Kit v3 (600-cycle format; Illumina MS-102-3003), paired-end, 600 bp sequencing was carried out on the Illumina Miseq.

The organisms from a metagenomic sample were classified using the metagenomics methodology from the illuminated basespace; after producing FASTQ files and demultiplexing indexed reads, the metagenomics workflow classes the reads. CosomsID (HUB), an online software solution that makes complicated metagenomic data analysis accessible, was used to analyze the metagenomics.

Statistical analysis

The gathered data were digitally processed and statistically examined using Stat Graphics Centurion version 19 and GraphPad Prism version 7. The ANOVA (F) test compared quantitative variables between the 3 groups and provided the mean and standard deviation (SD). The Pearson correlation coefficient was used to assess the qualitative variables' frequency and percentage (%). A statistically significant value was defined as a two-tailed P value ≤ 0.05.

The sample size was calculated using Open Epi according to the mean genus level of 0.67 in the control group and 3.08 in the cases group, and a cases-to-

control group ratio of 2:1. So, at a power of study of 80% and a confidence interval of 95%, the sample size was calculated to be 75 subjects, 25 in each group.

Results

A total of 75 participants were recruited: 25 were individuals without diabetes, 25 were patients with diabetes without nephropathy, and 25 were individuals with diabetes who had nephropathy (DN). Twenty-five healthy controls (12 women and 13 men) had a mean age of 45 ± 8.77 years. Of the 25 patients with diabetes, 11 were women, and 14 were men; their mean age was 45 ± 6.68 years. Of the 25 DN participants (10 women and 15 men), the mean age was 45 ± 6.68 years. The parameters of healthy subjects, diabetes patients, and DN patients included age, weight, Scr, BUN, serum albumin, urinary albumin creatinine ratio, and HbA1c. The clinical parameters of the subjects included age, weight, S.cr., BUN, serum albumin, urinary albumin creatinine ratio, and serum cholesterol. There were statistically significant differences among the studied groups regarding serum cholesterol, which statistically increased in the DN group (233.5 ± 20.76, p = < 0.0001***).

The studied groups had statistically significant differences regarding urinary albumin/creatinine ratio and fundus examination. Regarding the albumin creatinine ratio, Group III showed a statistically significant increase compared to other groups. (344.5 ± 614.2, p ≤ 0.001**). Also, there were statistically significant differences among the studied groups in urine analysis, with an increased frequency of albumin in urine in Group III compared to other groups. No statistically significant differences between BUN, S. creatinine, and serum electrolytes existed among the studied groups. Serum albumin was significantly decreased in the DN group (3.66 ± 0.51, p ≤ 0.0003***). Serum albumin was significantly decreased in the DN group (3.66 ± 0.51, p ≤ 0.0003***).

There were statistically significant differences among the studied groups regarding eGFR, albumin/creatinine ratio, and fundus examination (diabetic retinopathy). Regarding the albumin-creatinine ratio, Group III showed a statistically significant increase compared to other groups (p < 0.001). Also, there were statistically significant differences among the studied groups in urine analysis, with an increased frequency of albumin in urine among Group III compared to other groups (p < 0.004). There were statistically significant differences among the studied groups in diabetic retinopathy, which is present in Group III (p < 0.001). No statistically significant differences were found among the studied groups regarding BUN, serum creatinine, and serum electrolytes (Tab. 1). There was no statistical difference in alpha diversities between the different groups. There was a statistical difference in beta diversity between the control and diabetic nephropathy groups (p = 0.055) (Suppl. Tab. 1). The DN group exhibited significantly increased levels of Erysipelatoclostridium, Prevotella_9, and Escherichia coli compared to other groups, as shown in Figure 1. Escherichia-Shigella and Alistipes were shown to have a positive correlation with urinary albumin creatinine ratio (r = 0.88, p-value < 0.002) and (r = 0.91, p < 0.0001), respectively. The estimated glomerular filtration rate (eGFR) and [Ruminococcus] torques group had a negative correlation (r = –0.77, p-value < 0.0001). Cholesterol was positively correlated with Bacteroides (r = 0.99, p < 0.0001). Serum albumin was negatively correlated with Alistipes (r = –0.55, p = 0.004) (Tab. 2).

Discussion

Microalbuminuria, reduced creatinine clearance, and elevated serum creatinine are all part of the standard classical examination of DN [12]. However, an increase in the urine albumin-creatinine ratio does not necessarily correspond with a loss in renal function in individuals with diabetes [13].

An essential part of the etiology and progression of diabetes is gut microbial dysbiosis [14]. Increased levels of inflammatory cytokines and chemokines are associated with diabetes [15]. Thus, dysbiosis of the gut microbiota may contribute to an elevated inflammatory state in diabetes. Dysbiosis of the gut microbiota in diabetes may increase intestinal permeability [16].

Studies by Bäckhed et al. and Frost et al. [17, 18] have linked reduced diversity in the gut microbiota to reduced disease development. The microbiota in DN has already been studied. When Yu et al. [19] examined the gut microbiota composition of patients with diabetic kidney disease (DKD), they revealed that their microbiota was distinct.

We did not find any statistically significant differences in the alpha diversities of gut microbiota across the groups under investigation. However, our research did reveal statistically significant variations in beta diversity between the DN group and the control group. A lower level of gut microbial diversity was linked to DN. Our findings demonstrated the phylum-level distribution of bacteria in each group, with Proteobacteria and Bacteriodota predominating in the DN group. These findings are consistent with the findings of He et al. [20] who discovered that the gut microbial community of the DKD group differed significantly from that

Table 1. Comparison of Age, Weight, Sex, Duration of Diabetes, HbA1c, Serum Albumin, Cholesterol, Kidney Functions Tests, Serum Electrolytes, and Fundus Examination Among the Studied Groups

Variable	Group I (n = 25)	Group II (n = 25)	Group III (n = 25)	p
Age [years]	45 ± 8.77	45 ± 6.68	45 ± 6.68	0.37
Mean ± SD	27–58	32–57	32–57	
Weight [kg]				
Mean ± SD	80 ± 8.59	80 ± 7.59	80 ± 7.15	0.37
Range	66–95	60–93	68–92	
Sex				0.49
Male	N =13	N = 14	N = 15	
Female	N = 12	N = 11	N = 10	
Duration [years]				
Mean ± SD	.....	4.88 ± 0.97	8.61 ± 2.63	< 0.0001
Range		3–7	5–16	****
HbA1c: (%)				
Mean ± SD	4.56 ± 0.73	6.19 ± 0.74	7.04 ± 1.6	0.0001
Range	3.5–6	4.9–7.5	5–11	****
S. Albumin [g/dL]				
Mean ± SD	4.18 ± 0.54	4.28 ± 0.61	3.66 ± 0.51	< 0.0003
Range	3.5–5.1	3.5 – 5.2	3.1–5	***
Cholesterol [mg/dL]				
Mean ± SD	128.21 ± 27.06	188.31 ± 16.26	233.51 ± 20.76	0.0001
Range	80–185	154–218	200–280	****
eGFR [ml/min]				
Mean ± SD	106.41 ± 9.27	104.31 ± 9.81	93.71 ± 7.37	<0.0001***
Range	94–129	90–134	81–116	
BUN [mg/dL]	13 ± 4.01	14.34 ± 3.71	15.24 ± 5.19	0.19
Mean ± SD	7–20	8–20	7–25	
Range				
S. Creatinine [mg/dL]	0.88 ± 0.11	0.91 ± 0.11	0.94 ± 0.11	
Mean ± SD	0.7–1.1	0.8–1.1	0.8–1.1	0.1
Range				
Albu. creat. ratio [mg/g]				
Mean ± SD	19.71 ± 4.42	15.31 ± 5.56	344.51 ± 614.21	<0.001**
Range	10.2 – 27.9	10.2–27.9	42 – 2920	
S. Na [mEq/L]				
Mean ± SD	137.91 ± 2.36	137.21 ± 2.78	137.91 ± 3.16	0.61
Range	135–142	134–142	133–144	
S. K [mmol/L]				
Mean ± SD	4.07 ± 0.42	4.12 ± 0.49	4.11 ± 0.51	0.92
Range	3–4.8	3.4–4.9	3.4–5	
S. Cam [g/dL]				
Mean ± SD	9.49 ± 0.43	9.47 ± 0.34	9.44 ± 0.37	0.91
Range	8.9–10.4	8.9–10.1	8.9–10.1	
S. Po4 [mg/dL]				
Mean ± SD	3.67 ± 0.62	3.42 ± 0.63	3.68 ± 0.66	0.25
Range	2.6–4.6	2.5–4.7	2.6–4.6	

Variable	No	%	No	%	No	%	p
Fundus examination							
Normal	25	100	25	100	19	76	< 0.001**
Diabetic retinopathy	0	0	0	0	6	24	
Urine analysis							
Normal	25	100	25	100	16	64	< 0.004***
Abnormal	0	0	0	0	4	16	
Albumin +	0	0	0	0	5	20	
++							

Group I: control group; Group II: patients with type 2 diabetes without nephropathy, Group III: diabetic nephropathy; data expressed as mean ± SD or frequency (%); p- value < 0.05 was considered statistically significant. P — probability. *significance < 0.05. BUN — blood urea nitrogen; eGFR — estimated glomerular filtration rate; SD — standard deviation

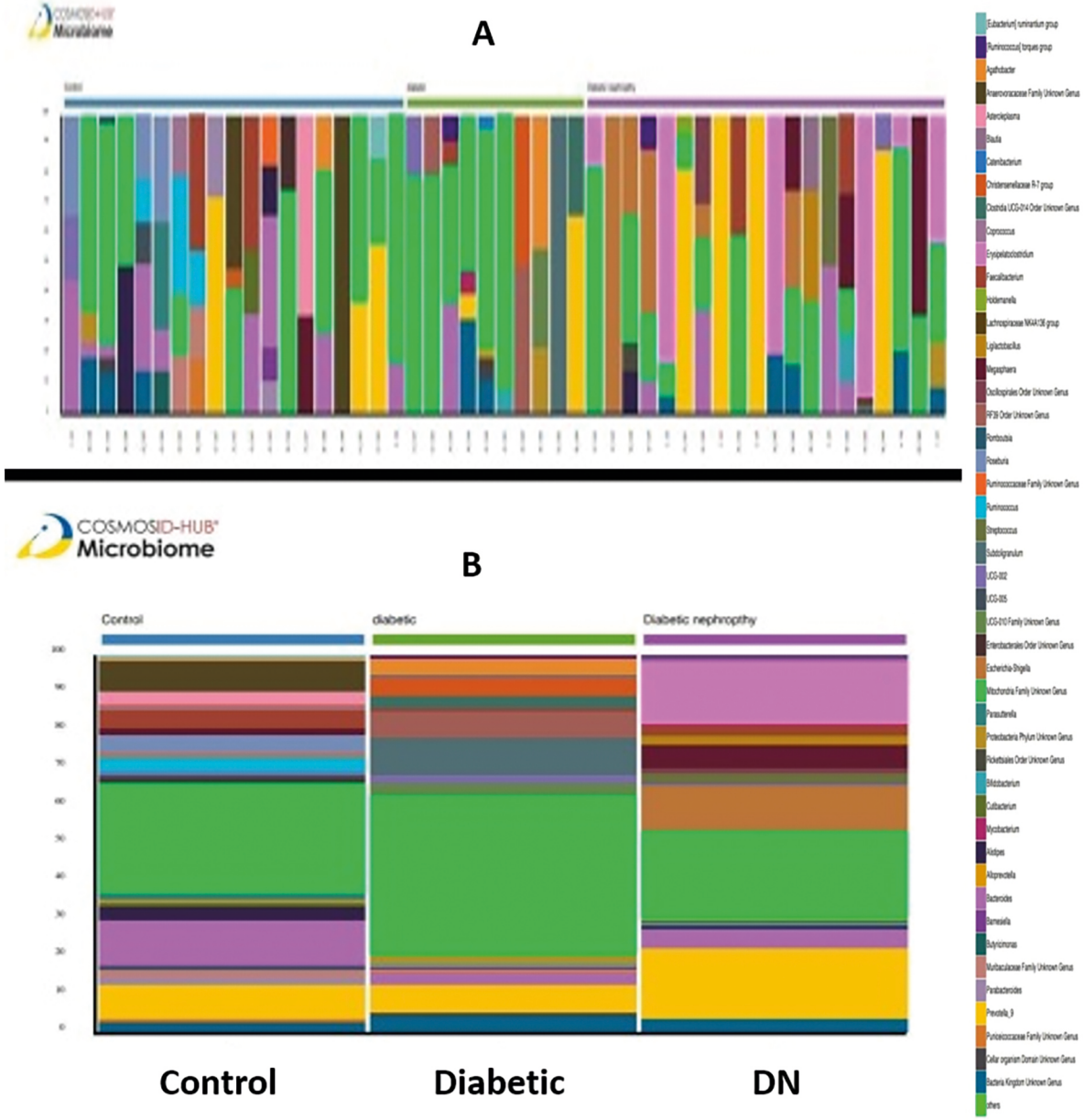

Figure 1. A. Stacked Bar Showing Bacterial Distribution in Genus Level Individual Samples of Each Group; **B.** Stacked Bar Aggregate by Cohort Showing Bacterial Distribution in Genus Level in Each Group

Table 2. Correlations Between Clinical Parameters and Gut Microbiota in DN Patients

Variable	eGFR		Cholesterol		Albumin creatinine ratio		S. albumin	
	r	P	r	P	r	P	r	P
Erysipelatoclostridium	-0.32	0.11	-0.17	0.42	-0.01	0.97	-0.03	0.87
Prevotella_9	0.17	0.42	0.21	0.32	-0.24	0.25	0.17	0.42
Escherichia-Shigella	0.23	0.26	-0.18	0.38	0.88	< 0.002**	-0.57	0.002
Bacteroides	-0.15	0.47	0.99	< 0.0001***	-0.18	0.38	-0.05	0.79
Alistipes	0.21	0.31	-0.17	0.41	0.91	< 0.0001***	-0.55	0.004**
[*Ruminococcus*] torques group	-0.77	< 0.0001***	0.28	0.18	-0.19	0.35	0.18	0.37

of the non-DKD group, with a notable increase in the phyla Proteobacteria and Bacteriodota. In gut microbial dysbiosis, proteobacteria are crucial for the host's nutritional condition, inflammation, and immunological and metabolic diseases [21]. Because proteobacteria have a detrimental effect on fat and glucose metabolism, they are also regarded as dangerous bacteria [22].

The current study compared 25 DN patients' gut microbiota and clinical characteristics. According to the findings, certain gut microbiota bacterial species had a positive correlation with clinical measures, while other gut microbiota bacterial species had a negative correlation. In line with Chen et al., serum albumin and *Alistipes* had a negative correlation [23].

Conclusions

Fecal samples from DN patients exhibit an imbalance in the gut microbiota, with an increase in *Erysipelatoclostridium*, *Prevotella_9*, and *Escherichia shigella* and a decrease in *Roseburia intestinalis*. An imbalance in the gut microbiota is significantly correlated with clinical indicators of renal function, cholesterol, blood albumin, and urine albumin creatinine ratio. The onset and course of DN may be predicted by the gut microbiome.

The study has some limitations. First, the study included only 75 participants, which may not be representative of the general population. Secondly, the study only collected data at a single time point, which may not capture the dynamic changes in the gut microbiota over time. Finally, the study did not provide mechanistic insights into how the gut microbiota influences the development of diabetic nephropathy.

Article information

Data availability statement

This study was conducted in the Department of Internal Medicine and Nephrology at Beni-Suef University, Egypt. This article includes all data generated or analyzed during this study.

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Author contribution

All authors reviewed and edited the manuscript and approved it for submission.

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Conflicts of interests

The authors declare no conflict of interest.

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