

Identification of the Alterations of Gut Microbiome and Its Relationship with NF- κ B in Patients with Type 2 Diabetes and Diabetic Peripheral Neuropathy

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Abstract

The surge in diabetic peripheral neuropathy (DPN) cases in recent years has underscored the urgent need for more intensive research into its prevention and management. The nuclear factor (NF)- κ B is recognized as a key mediator in the development of diabetic microvascular complications, with alterations in gut microbiota dynamics being a significant factor in triggering NF- κ B. Therefore, we proposed that there is a relationship among DPN, NF- κ B, and gut microbiota. We conducted 16S rRNA sequencing to analyze the gut microbiota of individuals with DPN and type 2 diabetes (T2DM), and healthy controls, while also measured NF- κ B levels. Our research findings indicate significant differences in the microbial composition among the three groups, particularly at the phylum level, with the control group showing a higher abundance of *Firmicutes* and a lower abundance of *Bacteroidetes* compared to the other two groups. Additionally, the α and β diversity were significantly lower in T2DM and DPN patients. Furthermore, a strong correlation was observed, indicating that the *Clostridiales* and *Trichospirillaceae* were significantly associated with levels of TG, FPG, and HDL-C. Moreover, specific gut bacteria, such as *Coccosia*, *Barnesiella*, and *Roseburia*, demonstrated robust associations with NF- κ B level, suggesting the potential role in the pathophysiology of DPN. Individuals afflicted with T2DM and DPN present with pronounced dysbiosis of the gut microbiota. This gut microbiota exhibits significant correlations with key physiological markers and clinical manifestations within the affected individuals, suggesting that these microbial shifts are integral to the pathogenesis and progression of diabetic complications.

Keywords

Diabetes; Diabetic peripheral neuropathy (DPN); Microbiome; NF- κ B

1. Introduction

In recent years, studies have shown that intestinal flora imbalance can affect the development of type 2 diabetes (T2DM) by causing metabolic disorders such as insulin resistance, and promote the growth of beneficial bacteria through probiotics supplementation, flora transplantation or other ways to reduce insulin resistance, and then treat diabetes [1]. The studies of the relationship between intestinal flora and diabetes mellitus is becoming increasingly popular, but the relationship between DPN patients and intestinal flora still needs to be further explored. At present,

an increasing number of studies have found a certain relationship between DPN and intestinal flora imbalance. Abnormal metabolites of intestinal flora play an important role in T2DM by affecting nutrient absorption, inducing chronic inflammation, regulating glucose and lipid metabolism, oxidative stress, and insulin resistance [2]. Imbalance in NF- κ B contributes to diabetes and is a major factor in the development and progression of DPN. The NF- κ B pathway has also previously been shown to be an important regulator of inflammatory damage in diabetic neuropathy [3]. In this study, 16S rRNA sequencing technology was used to analyze the distribution characteristics of intestinal flora in DPN patients further to understand the relationship between intestinal flora and DPN, hoping to establish a new and effective prevention and treatment for diabetic peripheral neuropathy by increasing attention to intestinal flora and further systematic research.

2. Materials and Methods

In this study, we recruited a total of 79 participants including healthy control (N = 26, the subjects did not have any disease), DPN (N = 26, patients with peripheral neuropathy associated with type 2 diabetes), and T2DM (N = 27, patients with type 2 diabetes alone), with each participant signing an informed consent form. General information was recorded for each person. This study was approved by the Institutional Ethics Committee of Hangzhou Lin'an Traditional Chinese Medicine (No. lazyyll20210918004). Serum NF- κ B levels were measured by ELISA. Fecal samples were collected for DNA extraction and 16S rRNA sequencing.

3. Results

3.1 16S rRNA sequence collation and analysis

Rarefaction analysis is used to indicate whether a high level of bacterial diversity has been obtained for subsequent analysis of collected fecal samples. The curve of each group reached saturation at 40,000 reads, indicating that the OTU diversity was almost completely covered. There was a total of 447 identical OTUs shared by the three groups. The largest overlap was observed between the Control and DPN groups, with 335 overlapping OTUs. On the other hand, the Control and T2DM groups had the fewest overlapping OTUs, with only 79 OTUs, which indicates the differences in microbial composition among the groups.

3.2 Analysis of taxonomic composition

To further analyze the composition of the intestinal flora, we used Qiime analysis to obtain taxonomic information for each sample. We constructed stack maps to visualize the proportions of microbial composition at different taxonomic levels. While most bacteria showed similar proportions among the three groups, there were significant differences observed for certain bacteria.

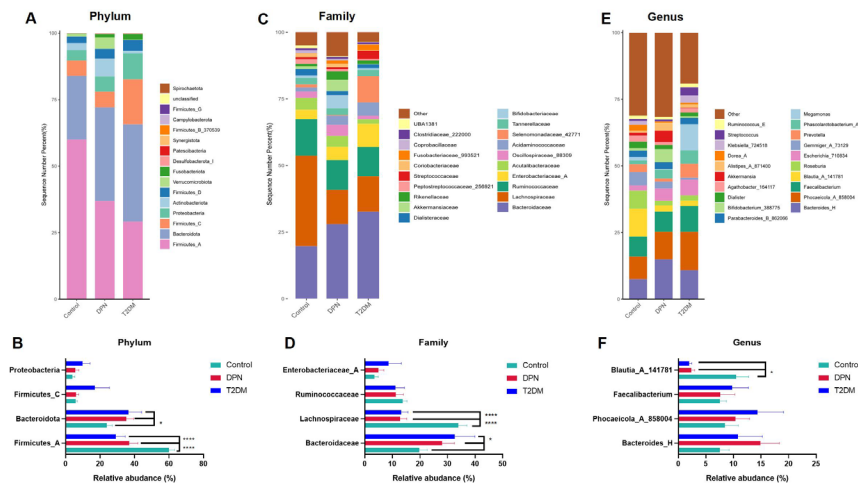


Figure 1. The stack maps of the proportions of microbial composition at different taxonomic levels. (The top 20 relative abundance of taxonomic composition in different sample sets at (A) phylum, (C) family, and (E) genus levels. Bar plot of the relative abundance of top 4 microbial bacteria at (B) phylum, (D) family, and (F) genus levels. Statistical significance was performed by T-test. *P < 0.05, ****P < 0.0001. Control, healthy control group; T2DM, type 2 diabetes mellitus group; DPN, diabetic peripheral neuropathy group.)

At the phylum level (Figures 1A and 1B), the relative abundance of *Bacteroidota* was significantly lower in the Control group compared to the other two groups. The relative abundance of *Firmicutes_A* was significantly higher in the Control group compared to the other two groups, and there was also a significant difference between the DPN and T2DM groups. At the family level (Figures 1C and 1D), the relative abundance of *Lachnospiraceae* was much higher in the Control group compared to the other groups, and the relative abundance of *Bateroidaceae* was significantly lower in the Control group compared to the other groups. At the genus level (Figures 1E and 1F), the relative abundance of *Blautia_A_141781* was significantly higher in the Control group compared to the other groups. Overall, these findings highlight the significant differences in microbial composition among the three groups.

3.3 Analysis of microbial species diversity

Significant differences in the abundance of specific bacteria were observed between different groups. At the phylum level, the bacteria with significant differences between Control and DPN are *Patescibacteria* and *Fusobacteriats*. The fecal samples of Control and T2DM patients were also compared, in which *Verrucomicrobiota*, *Firmicutes_C*, and *Fusobacteriota* had higher abundance and significant differences. Between patients with DPN and T2DM, *Firmicutes_C*, *Actinobacteria*, and *Verrucomicrobiota* showed significant differences. At the genus level, *Catenibacterium*, *Limosilactobacillus*, and *Ligilactobacillus* were significantly different between Control and DPN patients. *Alloprevotella* and *Klebsiella_724518* showed significant differences between Control and T2DM patients. In the comparison between T2DM and DPN patients, *Coprococcus_A_121497*, *Alloprevotella*, *Catenibacterium*, *UBA3402*, *AM51_8*, and *Ligilactobacillus* showed significant differences. LEfSe analysis further confirmed the distinct composition of characteristic microbiota in each group, with specific genera enriched in each group (Figures 2A and 2B). In summary, there are significant differences in the composition of intestinal flora between healthy control and patients with T2DM and DPN.

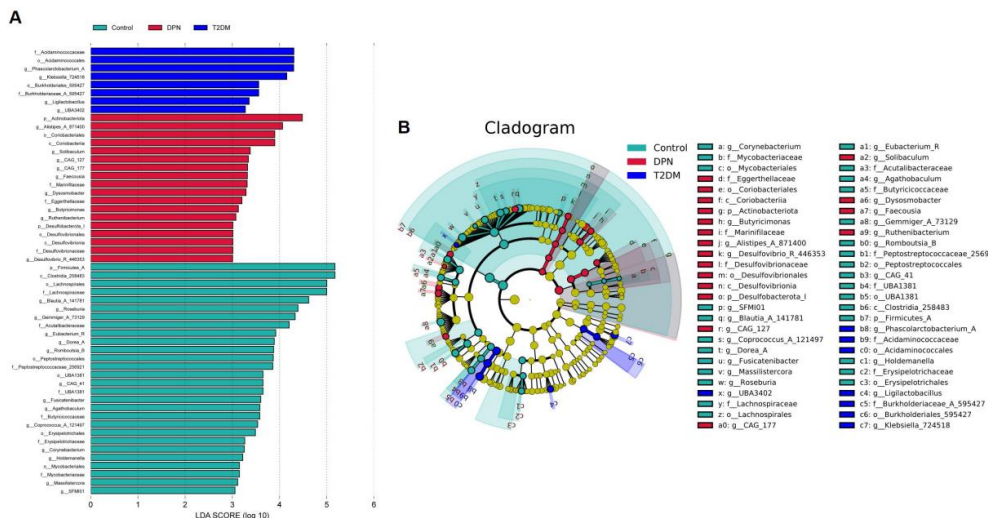


Figure 2. LEfSe analysis of microbial taxa enriched among three groups. [(A) LDA bar plot. Each bar indicates each bacteria. LDA set at 3. (B) Cladogram plot. From inside to the outside of the circle, the cladogram diagram corresponds to different taxonomic levels, and the lines between the levels represent the affiliation. Each circle node represents a species, with yellow indicating that there is no significant difference between groups, and not yellow indicating that the species is a characteristic microorganism of the corresponding color group.]

3.4 Alpha and Beta diversity analyses

As shown in Figures 3A-3D, α diversity was significantly lower in T2DM compared with the Control group with significant changes in chao1 and simpson index, suggesting reduced bacterial species in the intestines of individuals with type 2 diabetes. However, there was no significant difference in intestinal bacterial diversity between healthy Control and DPN patients. Additionally, Figures 3E and 3F showed differences in gut bacterial community structure between healthy Control and T2DM/DPN patients, with significant Bray-Curtis distances. However, there was no significant difference between T2DM and DPN patients.

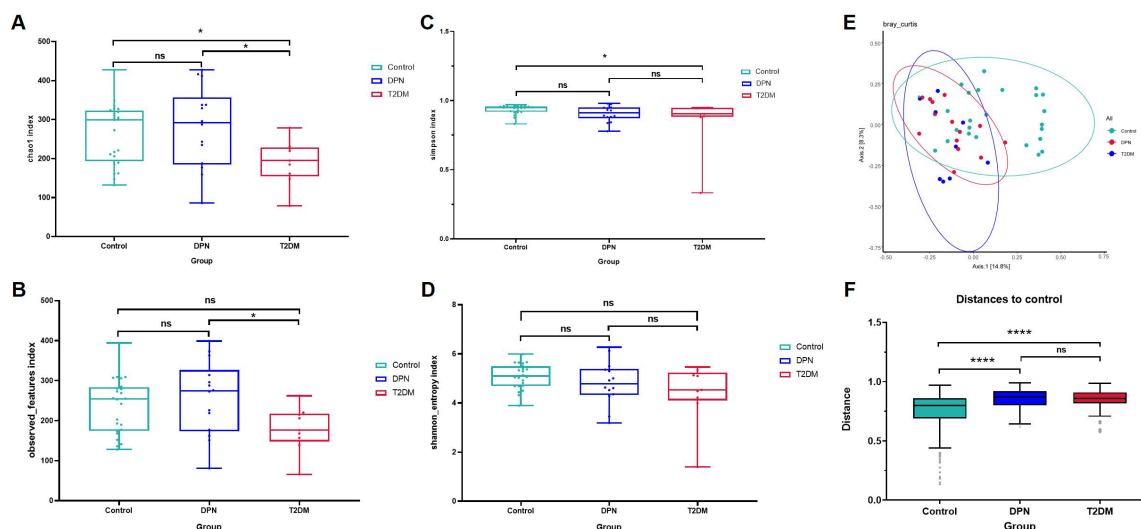


Figure 3. Alpha and Beta diversity analyses among three groups. [(A-D) Alpha diversity analysis estimated by (A) chao1 index, (B) observed features index, (C) Simpson index and (D) Shannon index. Statistical significance was performed by Kruskal Wallis analysis. (E-F) Beta diversity analysis estimated by (E) PCoA of OTUs and (F) distribution of Bray–Curtis distances of OTUs among T2DM, DPN, and Control. *P < 0.05, **P < 0.0001, ns, not significant.]**

3.5 Correlation between NF-κB or biochemical factors levels and intestinal bacteria

As shown in Figure 4A, the level of NF-κB was significantly decreased in T2DM patients compared with the Control group, however, there were no significant changes among other comparisons. At the phylum level (Figure 5B), NF-κB was significantly correlated with *Campylobacterota* and *Proteobacteria*. Furthermore, Figure 4C reveals a significant correlation between NF-κB and the genera *Caccamoeba*, *Barnesiella*, and *Roseburia*. Then, the heatmap in Figure 4C also clearly indicates the strong correlation between bacteria and biochemical factors.

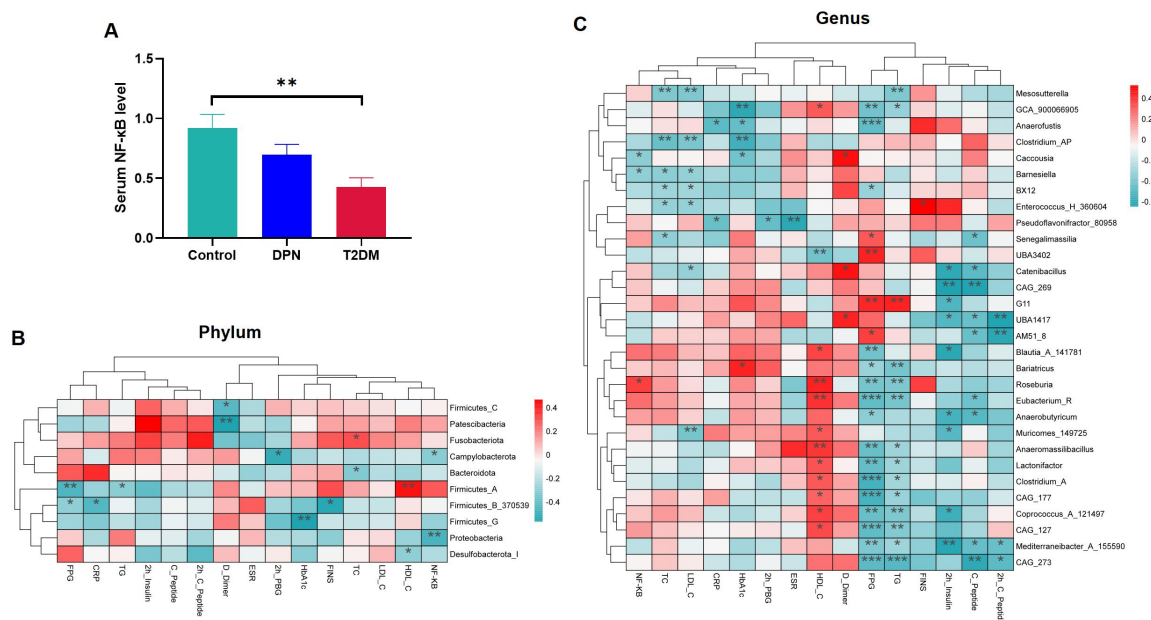


Figure 4. Correlation between NF-κB or biochemical factors levels and intestinal bacteria. [(A) Serum NF-κB levels determined by ELISA. (B) Correlation analysis between the microbiome composition and NF-κB or biochemical factors at phylum level. (C) Correlation analysis between the microbiome composition and NF-κB or biochemical factors at genus level. *P < 0.05, **P < 0.01, *P < 0.001.]**

4. Discussion

Our research reveals notable differences in gut microbiota composition among individuals with T2DM, DPN, and healthy controls. The T2DM group exhibits lower α -diversity, indicating a reduced variety of gut bacteria. β -diversity analysis highlights distinct microbial community structures between healthy individuals and those with T2DM or DPN. Furthermore, correlation analysis illustrates a strong link between bacteria and NF- κ B or biochemical factors, offering valuable insights into the impact of gut microbiota on host health.

Firmicutes and *Bacteroidetes* account for more than 90% of the total human intestinal flora. At the phylum level, we found the relative abundance of *Firmicutes_A* in healthy controls was significantly higher than that in T2DM and DPN groups, which is in accordance with previous study [4]. According to the results from correlation analysis, we also found *Firmicutes_A* is correlated with the level of FPG, TG and HDL-C. Further, some of the genera in *Firmicutes_A*, such as *Lachnospiraceae* and *Ruminococcaceae*, are known to ferment cellulose to produce short-chain fatty acids (SCFAs), especially acetate, propionate, and butyrate [5]. These SCFAs play an important role in maintaining intestinal health, regulating host immune response, and influencing energy metabolism [6]. In addition, reduced levels of SCFAs may affect gut hormone secretion, which in turn affects insulin sensitivity. In contrast to *Firmicutes_A*, we found *Bacteroidota* increased in relative abundance in T2DM and DPN patients.

The alpha diversity has delineated a more severe disruption in the gut microbiota diversity within the T2DM group and the Control group demonstrating a modestly enhanced gut community diversity in comparison to the DPN group. Our experiments consistently showed that gut microbiota diversity is most affected in uncomplicated diabetes, variations in DPN group diversity are likely due to a small sample size, an issue we aim to resolve in future studies for more reliable results. In DPN, it linked more to an increase in pathogenic bacteria than a decrease in beneficial ones. In contrast, pure diabetes is characterized by a reduction in beneficial bacteria without a corresponding rise in pathogenic bacteria [7]. This observation indirectly corroborates the significant reduction in gut microbiota diversity in the T2DM group and the increased diversity in the DPN group.

Beta diversity analysis reveals significant differences in microbial community structure between the Control group and the others, with no clear differences between the DPN and T2DM groups. The gut microbiota variations in diabetic patients are multifactorial. Numerous experiments underscore a significant correlation between alterations in gut microbiota and blood glucose levels in diabetic individuals [8-10]. The therapeutic interventions for T2DM and DPN patients also exert an impact on their gut microbiota composition.

We have observed a significant reduction in NF- κ B levels among patients with T2DM. NF- κ B is associated with both DPN and T2DM, with previous studies indicating that DPN patients exhibit higher serum NF- κ B levels compared to T2DM patients without neuropathy [11]. The role of NF- κ B in diabetic neuropathy is primarily linked to inflammatory responses and oxidative stress, where the activation of NF- κ B is a key factor in the pathogenesis of painful diabetic peripheral neuropathy [12]. During hyperglycemic conditions, there is a sustained increase in NF- κ B activity, and the neuroinflammatory response induced by elevated NF- κ B can activate microglia and astrocytes, further increasing the release of pro-inflammatory mediators and thus creating a vicious cycle of inflammatory response that intensifies pain sensation [13]. Moreover, dysbiosis of the gut microbiota may lead to an increased abundance of gram-negative bacteria, generating more endotoxins. Endotoxins can bind to toll-like receptor 4 (TLR4), subsequently activating the NF- κ B pathway [14]. In our study, we found associations between NF- κ B and the genera *Barnesiella* and *Roseburia*. Previous research has shown that inhibiting the phosphorylation of proteins associated with the NF- κ B signaling pathway can modulate the gut microbiota of *Barnesiella* species [15]. Activation of the NF- κ B signaling pathway has been shown to increase the relative abundance of *Roseburia* [16]. In addition to the known associations, these two types of gut bacteria have a close connection with diabetes mellitus type 2, with the abundance of *Barnesiella* being significantly negatively correlated with T2DM [17], and the abundance of *Roseburia* typically being lower in patients with T2DM [18]. Additionally, we have identified a correlation between *Coccousia* and NF- κ B, which has not been previously reported.

5. Conclusion

Individuals with T2DM exhibited a markedly reduced diversity of gut microbiota compared to healthy individuals, whereas patients with DPN showed no significant difference in gut bacterial diversity from healthy controls. Additionally, NF- κ B levels were significantly lower in those with T2DM, correlating with the abundance of intestinal flora. While this study offers novel insights into the interplay between gut microbiota and DPN, further investigation is required to confirm these results and elucidate the mechanisms through which gut microbiota influences DPN.

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Conflict of interest

There is no potential conflict of interest.

Data availability

Data will be made available on request.

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