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Research Article

Association between Gut Microbiome Dysbiosis and Diabetic Peripheral Neuropathy in Patients with Type 2 Diabetes Mellitus: A Multidisciplinary Cross-Sectional Study

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ABSTRACT

Background: Diabetic peripheral neuropathy is one of the most frequent chronic complication of type 2 diabetes mellitus and can result in pain, decreased sensation and a diminished quality of life. Recent findings indicate that the gut microbiome changes can play a role in metabolic inflammation, in compromised intestinal barrier function and nerve injury. There is, however, little local evidence to support the association between dysbiosis of the gut microbiota and DPN.

Objective: To determine the association between gut microbiome dysbiosis and diabetic peripheral neuropathy among patients with type 2 diabetes mellitus.

Methods: This multidisciplinary cross-sectional study was conducted at Gomal Medical College, Dera Ismail Khan, from January 2025 to June 2025. Total 76 type 2 diabetes mellitus (T2DM) adult patients were consecutively recruited by non-probability sampling method. Diabetic peripheral neuropathy was evaluated with Michigan Neuropathy Screening Instrument, 10-g monofilament and vibration perception and ankle reflex examination. Bacterial 16S ribosomal RNA gene analysis was performed using fresh stool samples. Alpha diversity was assessed by Shannon, Simpson and Chao1 indices and the composition of bacteria and gut dysbiosis index were compared between patients with and without neuropathy. Multivariable binary logistic regression was employed to determine factors associated with diabetic peripheral neuropathy independently.

Results: Diabetic peripheral neuropathy was identified in 42 of 76 patients, giving a frequency of 55.3%. Patients with neuropathy had a longer duration of diabetes, higher fasting blood glucose, higher HbA1c and increased C-reactive protein levels compared with patients without neuropathy. The Shannon diversity index was significantly lower in the neuropathy group than in the non-neuropathy group (2.71 ± 0.46 versus 3.18 ± 0.51; $p<0.001$), whereas the gut dysbiosis index was significantly higher (3.8 ± 1.3 versus 2.4 ± 1.0; $p<0.001$). Patients with neuropathy had reduced abundance of *Faecalibacterium*, *Roseburia*, *Akkermansia* and *Bifidobacterium* and increased abundance of *Escherichia–Shigella* and Proteobacteria. The gut dysbiosis index was positively correlated with the neuropathy score ($r=0.52$; $p<0.001$). In multivariable analysis, a higher gut dysbiosis index, lower Shannon diversity, longer duration of diabetes and higher HbA1c were independently associated with diabetic peripheral neuropathy.

Conclusion: Gut microbiome dysbiosis was significantly associated with diabetic peripheral neuropathy in patients with type 2 diabetes mellitus. Reduced microbial diversity, depletion of beneficial bacteria and increased abundance of potentially pro-inflammatory microorganisms may contribute to neuropathy through metabolic and inflammatory pathways. Longitudinal studies are needed to determine causality and evaluate microbiome-targeted interventions.

Keywords: Type 2 Diabetes Mellitus, Diabetic Peripheral Neuropathy, Gut Microbiome, Dysbiosis, Microbial Diversity, Inflammation.

INTRODUCTION

Type 2 diabetes mellitus is a large metabolic disorder which is associated with hyperglycemia and progressive decrease in beta cell function with insulin resistance. It is becoming more common, and causes significant numbers of microvascular and macrovascular complications globally. Diabetic peripheral neuropathy (DPN) is one of the most common and debilitating chronic complications of diabetes. Typically features numbness, burning sensation, tingling, loss of protective sensation and decreased ankle reflexes. Early recognition of factors associated with and potentially modifiable for foot ulceration, infection, gait disturbance and lower-limb amputation is clinically important as patients may be predisposed to these conditions due to progressive sensory loss [1-3].

Diabetic peripheral neuropathy is a complicated process with the following factors: prolonged hyperglycemia, oxidative stress, mitochondrial dysfunction, microvascular damage, advanced glycation end products and activation of inflammatory pathways. Other recognized risk factors, such as poor glycemic control and duration of diabetes, also do not account completely for the fact that some patients develop neuropathy and others do not, even though they have similar metabolic control. The gut microbiome has thus emerged as a likely source of complications associated with diabetes, and this has led to an increased focus on the microbiome in this area. The intestine contains many different microorganisms which form the intestinal microbiome, which takes part in nutrient metabolism, immune regulation, maintenance of the intestinal barrier and production of biologically active metabolites [4-6].

Gut microbiome dysbiosis is when there is an imbalance in the diversity, composition and function of the microbes. Dysbiosis in patients with type 2 diabetes has been linked to decreased levels of beneficial bacteria that produce short-chain fatty-acids and increased levels of gram-negative and potentially pro-inflammatory bacteria. Butyrate and other short chain fatty acids production is reduced and this can compromise the barrier function, while exposure to increased amounts of bacterial lipopolysaccharide could increase systemic inflammation. All these changes may exacerbate insulin resistance, endothelial dysfunction and oxidative stress. The peripheral nerves are vulnerable to metabolic and inflammatory injury, suggesting that disruption of the gut–metabolic–neural axis could play a role in the initiation and/or progression of diabetic peripheral neuropathy [7-10].

Previous studies have reported differences in intestinal microbial diversity and bacterial abundance between patients with diabetes with and without peripheral neuropathy. Lower microbial richness, depletion of *Faecalibacterium*, *Roseburia*, *Akkermansia* and *Bifidobacterium*, and increased abundance of Proteobacteria and *Escherichia–Shigella* have been described in metabolically unhealthy populations [11-14]. Nevertheless, evidence remains limited, particularly in South Asian populations, where dietary habits, medication exposure, ethnicity and environmental factors may produce distinct microbiome patterns. The present multidisciplinary cross-sectional study was therefore conducted to determine the association between gut microbiome dysbiosis and diabetic peripheral neuropathy among patients with type 2 diabetes mellitus at Gomal Medical College, Dera Ismail Khan. It also aimed to compare microbial diversity and bacterial composition between patients with and without neuropathy and to identify independent clinical and microbiome-related factors associated with diabetic peripheral neuropathy.

METHODOLOGY

This multidisciplinary cross-sectional study was conducted at Gomal Medical College, Dera Ismail Khan, from January 2025 to June 2025. The study was carried out through collaboration between the Departments of Medicine, Neurology, Pathology and Microbiology. Adult patients with established type 2 diabetes mellitus attending the medical outpatient department or admitted to the affiliated teaching hospital during the study period were assessed for diabetic peripheral neuropathy and gut microbiome dysbiosis. The cross-sectional design was selected to determine the association between gut microbial diversity and diabetic peripheral neuropathy at a single point in time. Written informed consent was obtained from all participants after explaining the study objectives, procedures, potential risks and confidentiality measures.

The study population were adult patients (30-75 years) diagnosed with type 2 diabetes mellitus for at least one year. Type 2 diabetes mellitus was determined by medical history, glucose lowering therapy or by known test

results of fasting blood glucose and glycated haemoglobin. Patients of both sexes willing to give a new stool sample and be clinically examined neurologically were included. Those who already had type 1 diabetes mellitus, gestational diabetes, acute diabetic complications, chronic gastrointestinal inflammatory disease, coeliac disease, chronic diarrhoea, gastrointestinal malignancy or major gastrointestinal surgery or gastrointestinal infection were excluded. Patients who had taken systemic antibiotics, probiotics, prebiotics or bowel-cleansing preparations in the previous 3 months were excluded as these were all likely to significantly impact gut microbe populations. Other exclusion criteria included patients with neuropathy caused by other conditions such as chronic alcohol abuse, hypothyroidism, advanced chronic kidney disease, B12 deficiency, chemotherapy, spinal cord disease, radiculopathy or hereditary neuropathy.

The sample size was determined using the single population proportion formula $n = Z^2p(1-p)/d^2$. Due to the lack of local data, an assumed proportion of 50% of patients with DPN with a dysbiosis of the gut microbiome was used to obtain the largest sample size anticipated. With a 95% confidence level, a Z value of 1.96, and an absolute precision of about 11.3% the minimum sample size calculated was 76 patients. Thus, 76 eligible patients of type 2 diabetes mellitus (T2DM) were recruited. All patients selected for the study, who had the necessary criteria for inclusion during the study period, were approached to join the study (consecutive non-probability sampling). After neurological evaluations, the subjects were divided into diabetic peripheral neuropathy and non-diabetic peripheral neuropathy group.

A structured data collection form was used to get Demographic and clinical data. Data included were age, sex, body mass index, smoking status, length of diabetes, diet, physical activity, medication use, use of insulin, use of metformin, recent antibiotic use and related comorbidities. BMI was determined by dividing weight (kg) by weight height (m2). Laboratory parameters included were fasting blood glucose, glycated haemoglobin, serum lipid profile, serum creatinine, estimated glomerular filtration rate, C-reactive protein and serum vitamin B12 (if available). The Michigan Neuropathy Screening Instrument (MNSI) was used to evaluate diabetic peripheral neuropathy (DPN). The assessment comprised a symptom questionnaire and inspection of the appearance of the feet, ulceration, ankle reflexes, vibration perception with a 128-Hz tuning fork and pressure sensation with a 10-g Semmes–Weinstein monofilament. The participants were classified into two groups: those with diabetic peripheral neuropathy, based on the instrument threshold and the clinical assessment. The participants were divided into two groups: DPN (predefined instrument threshold and clinical assessment). Total clinical score was also used to further classify neuropathy as mild, moderate and severe. A doctor or a neurologist assessed patients that had ambiguous results before the final diagnosis.

Fresh stool samples were collected from all participants in sterile, labelled, leak-proof containers and transported to the microbiology laboratory. Samples were processed promptly or stored at −80°C until analysis. Microbial DNA was extracted from stool using a commercial DNA extraction kit according to the manufacturer's instructions. The gut microbiome was assessed by amplification and sequencing of the bacterial 16S rRNA gene. Sequence data were subjected to quality control, taxonomic classification and microbial diversity analysis. Alpha diversity was assessed using the Shannon, Simpson and Chao1 indices, while beta diversity was evaluated using Bray–Curtis dissimilarity and principal coordinate analysis. The relative abundance of major bacterial groups was determined, and a gut dysbiosis index was calculated from the distribution of beneficial and potentially pathogenic bacteria. Laboratory personnel were blinded to the participants' neuropathy status.

All participants were asked to submit a new stool sample in a sterile, labeled, leakproof container. Samples were taken prior to any medication or diet changes and sent to the microbiology laboratory in a controlled manner. Stool samples were first kept at 2-8C and processed immediately or frozen at –80C until molecular testing. Using a validated commercial stool DNA extraction kit, we extracted microbial DNA following the manufacturer's protocol. The structure of the gut microbiota was analyzed by amplifying and sequencing the bacterial 16S ribosomal RNA (rRNA) gene. Quality control was carried out on sequence data followed by the taxonomic classification and the microbial diversity analysis. We used the Shannon, Simpson and Chao1 indices to measure alpha diversity and Bray–Curtis dissimilarity and principal coordinate analysis to measure beta

diversity. The abundance of major bacterial phyla and selected genera *(Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Faecalibacterium, Roseburia, Akkermansia, Bifidobacterium and Escherichia–Shigella)* was calculated relative to the other bacteria. The relative abundance of beneficial and potentially pathogenic bacteria groups was used to obtain a gut dysbiosis index. Assessment bias was minimized by blinding lab staff conducting the microbiome analysis from the participants' neuropathy status.

IBM SPSS Statistics version 26 was used to enter and analyse data. Normality was evaluated for continuous variables by the Shapiro–Wilk test and by looking at the histograms. Data were presented as mean ± SD and independent-samples t-test was used to compare normally distributed variables. Data on non-normally distributed variables was summarized as median and interquartile range, and compared with Mann–Whitney U test. Categorical variables were described as frequencies and percentages and compared using Fisher's exact test or the chi-square test, as appropriate. The diversity index of microbes and relative abundance of bacteria between the two groups (with and without DPN) were compared. The relationship between gut dysbiosis, microbial diversity, HbA1c and duration of diabetes and neuropathy scores was assessed using Pearson or Spearman correlation analysis. The variables with $p<0.20$ in the univariable analysis, in addition to clinically relevant confounding variables were included in the multivariable binary logistic regression model. Gut dysbiosis index, Shannon diversity index, age, sex, BMI, duration of diabetes, HbA1C, dietary fibre intake and antibiotic exposure were entered as independent variables, and DPN was entered as dependent variable. Odds ratios (95% confidence intervals) were reported and a p-value<0.05 was considered significant.

RESULTS

The study included 76 patients having Type 2 Diabetes Mellitus. The frequency of diabetic peripheral neuropathy was found to be 55.3% (42 patients) and 44.7% (34 patients) for those without peripheral neuropathy. The mean age of the participants was 55.8 ± 9.6 years, and 43 (56.6%) were male. The average duration of diabetes was 9.4 ± 5.1 years and the mean body mass index (BMI) was 28.1 ± 4.2 kg/m². Patients with diabetic peripheral neuropathy tended to be older and have a longer duration of diabetes and poor glycemic control when compared to those without diabetic peripheral neuropathy.

Table 1. Demographic and Clinical Characteristics According to Diabetic Peripheral Neuropathy Status

Variable	With neuropathy (n=42)	Without neuropathy (n=34)	p-value
Age, years	58.7 ± 8.8	52.2 ± 9.4	0.003
Male sex	25 (59.5%)	18 (52.9%)	0.565
BMI, kg/m²	28.6 ± 4.4	27.4 ± 3.9	0.216
Duration of diabetes, years	11.7 ± 5.0	6.6 ± 3.8	<0.001
Current smoking	10 (23.8%)	5 (14.7%)	0.321
Hypertension	28 (66.7%)	16 (47.1%)	0.085
Metformin use	31 (73.8%)	27 (79.4%)	0.567
Insulin therapy	22 (52.4%)	10 (29.4%)	0.044
Recent antibiotic exposure	11 (26.2%)	3 (8.8%)	0.050
Low dietary fiber intake	29 (69.0%)	13 (38.2%)	0.007

Values are presented as mean ± standard deviation or frequency and percentage.

The HbA1c level was also significantly elevated among those with neuropathy compared to those without neuropathy (9.1 ± 1.4% versus 7.8 ± 1.2%; $p<0.001$). Patients with neuropathy also had higher fasting blood glucose, triglyceride and C-reactive protein levels. Serum B12 levels were decreased in the neuropathy group but not statistically significantly. There was no difference in renal function between the two groups.

Table 2. Laboratory Characteristics of the Study Participants

Variable	With neuropathy (n=42)	Without neuropathy (n=34)	p-value
Fasting blood glucose, mg/dL	181.6 ± 42.3	151.8 ± 35.7	0.002
HbA1c, %	9.1 ± 1.4	7.8 ± 1.2	<0.001

Total cholesterol, mg/dL	197.4 ± 38.1	184.6 ± 34.7	0.133
LDL cholesterol, mg/dL	121.5 ± 31.6	111.2 ± 29.8	0.151
HDL cholesterol, mg/dL	39.2 ± 8.3	43.1 ± 9.0	0.054
Triglycerides, mg/dL	186.7 ± 62.4	153.5 ± 51.8	0.015
C-reactive protein, mg/L	5.9 ± 2.8	3.8 ± 2.1	0.001
Vitamin B12, pg/mL	298.6 ± 102.5	341.4 ± 109.8	0.083
Serum creatinine, mg/dL	1.08 ± 0.31	1.01 ± 0.26	0.295

There were 42 diabetic peripheral neuropathy patients, of which 19 (45.2%) were mild, 15 (35.7%) were moderate and 8 (19.0%) were severe. Patients with neuropathy had a mean Michigan Neuropathy Screening Instrument score of 6.7 ± 2.1, which was significantly higher than the 2.1 ± 1.0 score of patients without neuropathy ($p<0.001$). A total of 33 (78.6%) patients had abnormal monofilament sensation and 35 (83.3%) had impaired vibration perception. 29 (69.0%) patients had decreased or absent ankle reflexes.

Table 3. Peripheral Neuropathy Assessment Findings

Neuropathy variable	With neuropathy (n=42)	Without neuropathy (n=34)	p-value
MNSI examination score	6.7 ± 2.1	2.1 ± 1.0	<0.001
Burning or tingling sensation	34 (81.0%)	7 (20.6%)	<0.001
Numbness of feet	31 (73.8%)	5 (14.7%)	<0.001
Abnormal 10-g monofilament test	33 (78.6%)	4 (11.8%)	<0.001
Impaired vibration sensation	35 (83.3%)	6 (17.6%)	<0.001
Reduced or absent ankle reflex	29 (69.0%)	5 (14.7%)	<0.001

The gut microbiota analysis revealed that patients with DPN had significantly less alpha diversity. The mean Shannon diversity index was 2.71 ± 0.46 in the neuropathy group while the non-neuropathy group was 3.18 ± 0.51 ($p<0.001$). Likewise, there was a significant decrease in Chao1 richness index in patients with neuropathy. There was increased disruption of gut microbial community in the neuropathy group, as evidenced by having a higher gut dysbiosis index. Patients with neuropathy exhibited a decrease in the relative abundance of Faecalibacterium, Roseburia, Akkermansia and Bifidobacterium, which are known for their beneficial and short chain fatty acid producing properties. There was a significantly higher proportion of potentially pro-inflammatory bacteria, such as Escherichia–Shigella and Proteobacteria, in patients with neuropathy in comparison. The Firmicutes/ Bacteroidetes ratio was also significantly increased in the neuropathy group.

Table 4. Comparison of Gut Microbiome Indicators between Study Groups

Microbiome variable	With neuropathy (n=42)	Without neuropathy (n=34)	p-value
Shannon diversity index	2.71 ± 0.46	3.18 ± 0.51	<0.001
Simpson diversity index	0.78 ± 0.07	0.84 ± 0.06	<0.001
Chao1 richness index	188.6 ± 42.7	224.9 ± 47.3	0.001
Gut dysbiosis index	3.8 ± 1.3	2.4 ± 1.0	<0.001
Firmicutes-to-Bacteroidetes ratio	1.91 ± 0.72	1.46 ± 0.55	0.004
Faecalibacterium, %	4.6 ± 2.0	6.5 ± 2.4	<0.001
Roseburia, %	2.3 ± 1.1	3.2 ± 1.4	0.003
Akkermansia, %	1.1 ± 0.7	1.7 ± 0.9	0.002
Bifidobacterium, %	2.8 ± 1.3	3.7 ± 1.5	0.007
Escherichia–Shigella, %	4.9 ± 2.5	2.9 ± 1.8	<0.001
Proteobacteria, %	9.8 ± 4.1	6.7 ± 3.2	0.001

The gut dysbiosis index was significantly associated with the Michigan Neuropathy Screening Instrument score ($r=0.52$, $p<0.001$), HbA1c level ($r=0.41$, $p<0.001$) and duration of diabetes ($r=0.36$, $p=0.002$), as indicated by correlation analysis. There was a negative correlation between Shannon index and neuropathy score ($r=-0.47$, $p<0.001$),

suggesting that the lower the microbial diversity, the higher the severity of neuropathy. The abundance of Faecalibacterium was negatively correlated with neuropathy score (r=−0.39, p=0.001), while Escherichia–Shigella had a positive correlation (r=0.43, p<0.001).

Table 5. Correlation of Microbiome Indicators with Clinical Variables

Variables	Correlation coefficient	p-value
Gut dysbiosis index and MNSI score	0.52	<0.001
Gut dysbiosis index and HbA1c	0.41	<0.001
Gut dysbiosis index and diabetes duration	0.36	0.002
Shannon index and MNSI score	−0.47	<0.001
Faecalibacterium abundance and MNSI score	−0.39	0.001
Escherichia–Shigella abundance and MNSI score	0.43	<0.001

All independent factors associated with diabetic peripheral neuropathy were derived by the use of multivariablestatistical logistic regression. The gut dysbiosis index was independently associated with neuropathy after adjustment for age, sex, BMI, length of diabetes, HbA1c, antibiotic use and dietary fiber intake. For each increase of one unit in the dysbiosis index, the risk of diabetic peripheral neuropathy rose by ~2-fold. Other factors independently associated with neuropathy were lower Shannon diversity, longer diabetes duration and higher HbA1c.

Table 6. Multivariable Logistic Regression Analysis of Factors Associated with Diabetic Peripheral Neuropathy

Variable	Adjusted odds ratio	95% confidence interval	p-value
Gut dysbiosis index, per unit increase	2.08	1.24–3.49	0.006
Shannon diversity index, per unit increase	0.31	0.11–0.88	0.028
Duration of diabetes, per year	1.16	1.04–1.30	0.008
HbA1c, per 1% increase	1.72	1.13–2.62	0.011
Age, per year	1.04	0.97–1.11	0.262
Low dietary fiber intake	2.11	0.71–6.29	0.179
Recent antibiotic exposure	2.46	0.60–10.13	0.212

Overall, the findings demonstrated that diabetic peripheral neuropathy was associated with reduced gut microbial diversity, increased intestinal dysbiosis, depletion of beneficial short-chain fatty-acid-producing bacteria and greater abundance of potentially pro-inflammatory microorganisms. These associations remained significant after adjustment for important clinical and metabolic confounding factors.

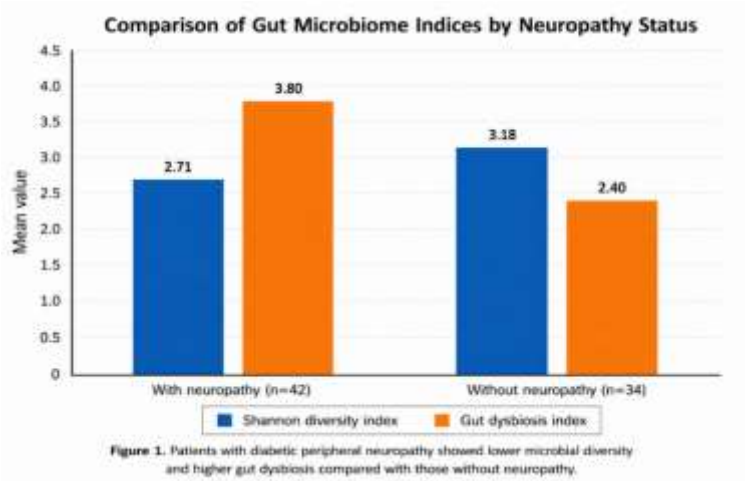

Figure 1. Comparison of Gut Microbiome Indices by Neuropathy Status.

Patients with diabetic peripheral neuropathy showed significantly lower Shannon diversity index and higher gut dysbiosis index compared with patients without neuropathy, indicating reduced microbial diversity and greater intestinal dysbiosis in the neuropathy group.

DISCUSSION

The present study demonstrated a significant association between gut microbiome dysbiosis and diabetic peripheral neuropathy among patients with type 2 diabetes mellitus. Patients with neuropathy had lower Shannon, Simpson and Chao1 diversity indices, together with a higher gut dysbiosis index, than those without neuropathy. They also showed depletion of potentially beneficial bacteria, including *Faecalibacterium*, *Roseburia*, *Akkermansia* and *Bifidobacterium*, with increased abundance of *Escherichia–Shigella* and Proteobacteria. These findings are consistent with a 2020 clinical study that reported distinct intestinal microbial characteristics in patients with type 2 diabetes complicated by peripheral neuropathy, including changes in microbial diversity, richness and bile-acid-related bacterial profiles [1]. A large population-based analysis also found that lower microbial richness and Shannon diversity were associated with insulin resistance and type 2 diabetes, supporting the concept that reduced microbial diversity reflects impaired metabolic health [15].

This decrease in short-chain fatty-acid-producing bacteria in the gut could be a biological explanation of the link between dysbiosis and peripheral nerve damage in neuropathy patients. Faecalibacterium and Roseburia are key species that produce butyrate, which plays a key role in intestinal barrier integrity, immune regulation and control of systemic inflammation. A decline in these organisms can lead to a breakdown of the intestinal barrier and allow the bacterial products (lipopolysaccharide) to enter the blood. Another multiethnic cohort study found lower abundance of the short-chain fatty-acid (SCFA)-producing genera and higher abundance of gram-negative endotoxin-producing bacteria as well as elevated level of lipopolysaccharide-binding protein (LBP) in those with type 2 diabetes, which indicates a relationship between dysbiosis, bacterial translocation and chronic inflammation [16]. Likewise, a study of prediabetes and diabetes in a population revealed changes in the populations of bacteria that produce butyrate and changes in butyrate-producing bacterial metabolic pathways associated with glucose intolerance [17].

The patients with DPN in the present study were found to have higher levels of HbA1c, fasting glucose, triglyceride and C-reactive protein than those without neuropathy. Moreover, the gut dysbiosis index was positively associated with HbA1c and severity of neuropathy, while the Shannon diversity index was negatively associated with the score of neuropathy. The results indicate that there may be a contribution from both poor glycemic control and systemic inflammation and microbiome disruption to peripheral nerve injury. Hyperglycemia itself contributes to oxidative stress, mitochondrial dysfunction and advanced glycation and microvascular impairment; and inflammatory signals coming from the microbiome can also activate immune and neuro-inflammatory pathways. Large-scale studies have demonstrated gut microbial characteristics link to T2D and metabolic risk factors, metabolites in circulation after accounting for common clinical factors [18]. Another study of the microbiome in Pakistan also found ethnic-specific gut microbial signatures in type 2 diabetes, highlighting that ethnicity, diet, medication exposure and environment may have a role in shaping the microbiome seen in living in Pakistan [19].

The present findings are further supported by emerging experimental and interventional evidence. In a 2023 study, gut microbiota transplant from the mice with diabetic distal symmetric polyneuropathy (DSPN) led to more severe neuropa-thological abnormalities in diabetic mice.Transplantation of gut microbiota from the mice with diabetic distal symmetric polyneuropathy (DSPN) promoted more severe neuropa-thological abnormalities in diabetic mice in a 2023 study. Fecal microbiota transplantation from healthy donors was shown to benefit clinical outcomes related to neuropathy in patients with distal symmetric polyneuropathy in the clinical component of the same investigation, indicating that a disturbance in the gut flora may not only be associated but also be a contributing cause [20]. Experimental studies reported in 2023 also confirmed abnormal intestinal microbiota in diabetic peripheral neuropathy models, which is associated with neural dysfunction, suggesting a connection between dysbiosis, inflammation and neural dysfunction [12]. However, diabetes peripheral neuropathy has several causes, and known causes like age, diabetes duration, glycemic control, obesity,

dyslipidaemia, renal disease and vascular disease are important. The fact that the gut dysbiosis index remained an independent factor also after adjusting for multiple confounders in the present study indicates that microbiome disruption may be a pathway of neuropathy other than the traditional pathways.

There are some limitations of this study. The cross-sectional nature of the study does not allow for temporal sequence or causality to be established; it is not possible to determine if the gut dysbiosis led to neuropathy or if it was a result of severity of diabetes, dietary changes, medication or gastrointestinal dysfunction. Limits to generalizability of results may also exist because of the relatively small sample size (76 patients) from a single institution. The composition of the gut microflora may be influenced by dietary habits, geographical location, metformin, antibiotics, proton pump inhibitors (PPIs) and residual confounding after statistical adjustment. Furthermore, 16S ribosomal RNA (rRNA) sequencing offers mainly taxonomic data and is limited in its capacity to describe microbial genes, metabolites and the functional activity of microbial species. Larger sample sizes, repeated stool samples, shotgun metagenomic sequencing and short-chain fatty acids, bile acids, and circulating inflammatory markers should be measured in future multicentre, longitudinal studies. Additionally, carefully planned diet, probiotic and postbiotic, as well as fecal microbiota transplantation (FMT) studies are required to assess if gut microbiome manipulation has a role in preventing or treating diabetic peripheral neuropathy.

CONCLUSION

Gut microbiome dysbiosis was significantly associated with diabetic peripheral neuropathy in patients with type 2 diabetes mellitus. Patients with neuropathy demonstrated reduced microbial diversity, greater gut dysbiosis, depletion of beneficial short-chain fatty-acid-producing bacteria and increased abundance of potentially pro-inflammatory microorganisms. Higher gut dysbiosis, poor glycemic control and longer duration of diabetes were independently associated with neuropathy, while greater microbial diversity appeared to have a protective association. These findings support a possible microbiota–inflammation–nerve axis in the development of diabetic peripheral neuropathy. However, longitudinal and interventional studies are required before gut microbiome assessment or microbiome-targeted therapies can be recommended for routine clinical practice.

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