Original Article

Gut Microbiota in Type 2 Diabetes Individuals and Correlation with Monocyte Chemoattractant Protein1 and Interferon Gamma from Patients Attending a Tertiary Care Centre in Chennai, India

Premalatha Pushpanathan, Padma Srikanth, Krishna G. Seshadri[1], Sribal Selvarajan, Ravi Shankar Pitani[2], Thomas David Kumar[3], R. Janarthanan[3]

Departments of Microbiology, [1]Endocrinology and [2]Community Medicine, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, [3]Shrimpex Biotech Services India Private Limited, Sholinganallur, Chennai, Tamil Nadu, India

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) and obesity are associated with changes in gut microbiota and characterized by chronic low-grade inflammation. Monocyte chemoattractant protein-1 (MCP-1) and interferon gamma (IFNγ) are proinflammatory cytokines which play an important role in the development of T2DM. We undertook this study to analyze the gut microbiota of T2DM and nondiabetic subjects and to determine the profile of MCP 1 and IFNγ in the same subjects attending a tertiary care center in Chennai, Tamil Nadu, India. **Methods:** The study included 30 subjects with clinical details. Stool and blood samples were collected from all the subjects. DNA was extracted from fecal samples and polymerase chain reaction was done using fusion primers. Metagenomic analysis was performed using ion torrent sequencing. The reads obtained were in FASTA format and reported as operational taxonomic units. Human MCP 1 and IFNγ enzyme linked immunosorbent assay (ELISA) were performed for 23 serum samples. **Results:** The study consisted of 30 subjects; 17 were T2DM and 13 were nondiabetics. The gut microbiota among T2DM consisted predominantly of Gram negative bacteria; *Escherichia* and *Prevotella*, when compared with the nondiabetic group with predominantly Gram positive organisms suchas *Faecalibacterium*, *Eubacterium,* and *Bifidobacterium*. The mean MCP-1 values in the diabetic group were 232.8 pg/ml and in the nondiabetic group 170.84 pg/ml. IFNγ (mean 385.5 pg/ml) was raised in glycated hemoglobin (HbA1c) group of 6.5–7.5% which was statistically significant. Association of *Escherichia* with T2DM and association of *Bifidobacteria* in the nondiabetics were also statistically significant. **Conclusion:** *Escherichia* counts were elevated in T2DM with HbA1c of 6.5–8.5% which was statistically significant suggesting that lipopolysaccharides present in the cell wall of Gram-negative bacteria may be responsible for low-grade inflammation as evidenced by elevated MCP-1 and IFNγ levels in T2DM with the same HbA1c levels.

Key words: Gut microbiota, glycated hemoglobin, interferon gamma, monocyte chemoattractant protein-1, metagenomic sequencing, nondiabetes, type 2 diabetes mellitus

Corresponding Author: Dr. Padma Srikanth, Professor, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai - 600 116, Tamil Nadu, India.
E-mail: srikanth_padma@rediffmail.com

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.183474

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by a combination of hereditary and environmental

Cite this article as: Pushpanathan P, Srikanth P, Seshadri KG, Selvarajan S, Pitani RS, Kumar TD, *et al*. Gut Microbiota in Type 2 Diabetes Individuals and Correlation with Monocyte Chemoattractant Protein1 and Interferon Gamma from Patients Attending a Tertiary Care Centre in Chennai, India. Indian J Endocr Metab 2016;20:523-30.

factors.[1] Obesity is the primary cause for obesity-linked insulin resistance.[2] Obesity and diabetes mellitus are characterized by a state of low grade inflammation.[2] As of 2014, 387 million people globally are diabetic[3] and more than 1.9 billion are overweight of which 600 million are obese.[4] India accounts for 66.84 million diabetics and stands second to China which accounts for 96.28 million.[5] Ineffective insulin use and lifestyle changes lead to systemic complications and mortality in diabetics.

Recently, changes in gut microbiota have been implicated in the development of T2DM and obesity.[6] Gut microbiota affects energy balance,[7,8] glucose metabolism,[9,10] and low-grade inflammation[11] associated with obesity and T2DM.

We undertook this study to determine the composition of gut microbiota in T2DM and nondiabetic subjects and to identify alterations in gut microbiota using metagenomic sequencing as well as to explore the role of monocyte chemoattractant protein-1 (MCP-1) and interferon gamma (IFNγ) in contributing to low-grade chronic inflammation in T2DM.

Methods

Subjects and sample collection

This study protocol was approved by the Ethical Committee of Sri Ramachandra University. Informed written consent was obtained from all the subjects enrolled in the study. The study included 17 subjects diagnosed with T2DM and 13 nondiabetics as confirmed by the physician and glycated hemoglobin (HbA1c).

Details such as past history, personal and treatment history, HbA1c, fasting blood sugar, postprandial blood sugar, body mass index (BMI), and diet of the participants were recorded in a proforma. The inclusion criteria for the study were subjects with T2DM whose HbA1c was > 6.5% and nondiabetics willing to participate with HbA1c < 6.5%. Exclusion criteria included type 1 diabetes mellitus, personal history of smoking and alcohol consumption, treatment with antibiotics in the preceding 3 months, chronic inflammatory diseases, use of corticosteroids, breastfeeding or pregnancy.

Sample collection

A total of 30 fecal samples (17 T2DM and 13 nondiabetics) were collected in a sterile container, brought to the laboratory, and kept at + 4°C until processing. In addition, 23 (12 T2DM and 11 nondiabetics) serum samples were collected from the same subjects in gel-based, yellow-capped vacutainer tubes (Becton and Dickinson, USA). All samples were coded giving a specific laboratory coding.

T2DM subjects were categorized according to HbA1c as (6.5–7.5% n = 7, 7.6–8.5% n = 5, and >8.5% n = 5). BMI was also used to categorize the T2DM subjects as normal BMI (18–23) n = 1, overweight (23–28) n = 5, and obese (>28) n = 11.

Nondiabetic subjects with normal BMI were n = 4, overweight n = 5, and obese n = 4.

Fecal DNA extraction

DNA extraction was done using QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) as per the manufacturer's protocol.

Briefly, 220 mg (approximately) of stool was taken in a microcentrifuge tube to which 1 ml of InhibitEX buffer was added and vortexed until samples were thoroughly homogenized and centrifuged at 14,000 rotations per minute (rpm) for 1 min. Proteinase K (25 µl) was added in a new centrifuge tube to which 600 µl of the supernatant from the previous step was added. Lysis buffer (600 µl) was added to the above mixture, vortexed for 15 s, and incubated at 70°C for 10 min. Ethanol (600 µl) was added to the lysate, vortexed, and centrifuged at 14,000 rpm for 1 min. The supernatant was transferred to spin column, centrifuged until all the lysate was loaded on the column. Wash buffer (500 µl) 1 and 2 were added subsequently, centrifuged at 14,000 rpm for 1 and 3 min, respectively. The spin column was transferred to a new collection tube and spun at 14,000 rpm for 2 min. Spin column was then transferred to a new microcentrifuge tube, and 200 µl of elution buffer was added, incubated at room temperature for 3 min, and centrifuged at 14,000 rpm for 1 min for obtaining DNA. The eluted DNA was aliquoted and stored immediately at − 20°C until polymerase chain reaction (PCR) was performed.

Polymerase chain reaction amplification

PCR was performed for the extracted DNA using Qiagen multiplex PCR mastermix (Qiagen) with Fusion primers [Tables 1], and PCR carried out [Table 2a and 2b].

Polymerase chain reaction product purification

PCR product purification was done by carboxylic coated magnetic beads. The purified PCR product size and quantity were verified by Agilent bioanalyzer 2100. Based on the result obtained the PCR product was diluted to 26 pmol/µl and used for metagenomic sequencing.

Metagenomic sequencing

Metagenomic sequencing was performed for all the fecal sample's PCR products using Ion Torrent PGM (Ion Torrent Personal Genome Machine) sequencing platform.

Methodology

Adapters were ligated to the ends of DNA fragments during PCR. The fragments were clonally amplified onto ion sphere particles. The ion sphere particle coated with the template was applied to Ion chip and deposited in chip wells which were then loaded into PGM machine and sequencing run setup.

Analysis

NCBI 16S database was chosen for analysis of the reads. Reads <60 bases were excluded, and percentage of the match for grouping similar sequence levels was set at 90%. The reads were clustered into operational taxonomic units at 90% level. The results were displayed in the form of a pie chart and used for further analysis.

Enzyme linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed for MCP-1 and IFNγ using Peprotech mini ELISA development kit (Peprotech, NJ, USA) as per manufacturer's protocol for all the available 23 serum samples. Readings for MCP-1 and IFNγ were taken using an ELISA plate reader (Thermoscientific Multiskan EX) monitored at 5 min interval for 20 min and 10 min, respectively, at 405 nm with reference range at 650 nm. The quantity of the test sample was measured using the curve generated by the standard concentrations of each calibrator using the graph provided by the manufacturer.

Statistical analysis

Statistical analysis was done using SPSS software version 16.0 (IBM SPSS Pvt. Ltd.,) and R software 3.0.2. (The R foundation).

Results

Among the subjects enrolled 14 were male and 16 were female, the age ranged from 22 to 70 years. T2DM subjects were grouped based on HbA1c into three categories - 6.5–7.5% (n = 7), 7.6–8.5% (n = 5), and > 8.5% (n = 5); the BMI ranged from 20.2 to 38.8.

Nondiabetics (HbA1c < 6.5%) were categorized based on BMI into normal weight (n = 4), overweight (n = 5), and obese (n = 4) [Tables 3a and b].

Most of the subjects were from Chennai (67%), and neighboring districts in Tamil Nadu (17%), three from Andhra Pradesh (10%), and two from North-East India (6%). All the subjects were nonsmokers and nonalcoholics, with no history of intake of antibiotics/probiotics in the preceding 3 months and gave no history of steroid therapy.

Table 1: V6 fusion primer sequence for polymerase chain reaction

Primer details	Nucleotide sequence
Adapter sequence forward primer (primer A-key)	5'-CCATCTCATCCCTGCGTGTCTCCG ACTCAG-GAATTGACGGGGRCCC-3'
Adapter sequence reverse primer (primer P1-key)	5'-CCTCTCTATGGGCAGTCGGTGAT-GGGTTGCGCTCGTTRC-3'

Table 2a: Reaction mix for polymerase chain reaction

Reaction mix	Volume
Master mix	10 µl
Fusion primer	2 µl
DNA template	2 µl
Water	6 µl
Total	20 µl

Table 2b: polymerase chain reaction cycling condition

Steps	Temperature and duration
Initial denaturation	95°C for 15 min
Denaturation	95°C for 30 s
Annealing	56°C for 1 min
Extension	72°C for 1 min
Number of cycles	40
Final extension	72°C for 5 min

Table 3a: Profile of type 2 diabetes mellitus subjects

Subject ID	Age (years)	HbA1C	BMI
1	50	9.4	33.48
2	70	8.1	31.7
3	52	7.8	37.2
5	67	16.7	20.16
6	22	8.3	25.55
7	45	6	27.06
8	75	7.2	25.62
12	48	9.3	33.7
13	58	6.5	24.03
14	39	6.5	28.9
16	41	7.2	38.79
17	69	8	33.82
18	52	6.5	31.72
19	58	6.5	37.54
21	49	7.6	31.61
24	30	13	30.38
25	51	10.9	23.89

BMI: Body mass index (Kg/m^2), HbA1C: Glycated hemoglobin (%)

Table 3b: Profile of nondiabetics (control)

Subject ID	Age (years)	HbA1C	BMI
4	45	5.5	25.82
9	31	5.8	22.79
10	35	5.6	28.67
11	34	5.5	26.33
15	46	5.5	32.94
20	63	5.9	20.05
22	51	5.8	27.66
23	42	5.6	28.95
26	28	5.6	29.77
27	29	5.7	23.38
28	26	5	26.21
29	26	5.4	21.25
30	40	5.7	22.42

BMI: Body mass index (Kg/m^2), HbA1C: Glycated hemoglobin (%)

Analysis of gut microbiota at the phyla level showed Firmicutes (49%), Proteobacteria (32%), Bacteroidetes (17%), and Actinobacteria (2%) among T2DM and Firmicutes (49%) Proteobacteria (26%), Bacteroidetes (18%), and Actinobacteria (7%) among nondiabetics [Figure 1a and b]. However, there were differences observed between T2DM and nondiabetics at the genera level. The predominant phyla encountered in T2DM was Proteobacteria, composed of *Escherichia*, *Citrobacter*, and *Acinetobacter*. Gram positive bacteria such as Firmicutes which comprises of *Faecalibacterium*, *Eubacterium*, *Clostridium*, and *Bifidobacterium* of Actinobacteria were encountered in nondiabetics. Among the T2DM *Escherichia* (Gram-negative bacteria) was predominant and in the nondiabetics *Faecalibacterium* followed by *Eubacterium* and *Bifidobacterium* were predominant [Table 4]. *Escherichia* was part of gut microbiota in 58.8% of T2DM subjects and 7.7% of nondiabetics ($P = 0.007$, odds ratio = 7.6). Apart from *Escherichia*, *Lactobacillus* was also found to be 35.3% in T2DM and 7.7% in nondiabetics. *Bifidobacterium* was found predominantly among the nondiabetic 38.5% ($P = 0.04$ using Fisher's Exact Chi square test) [Table 5].

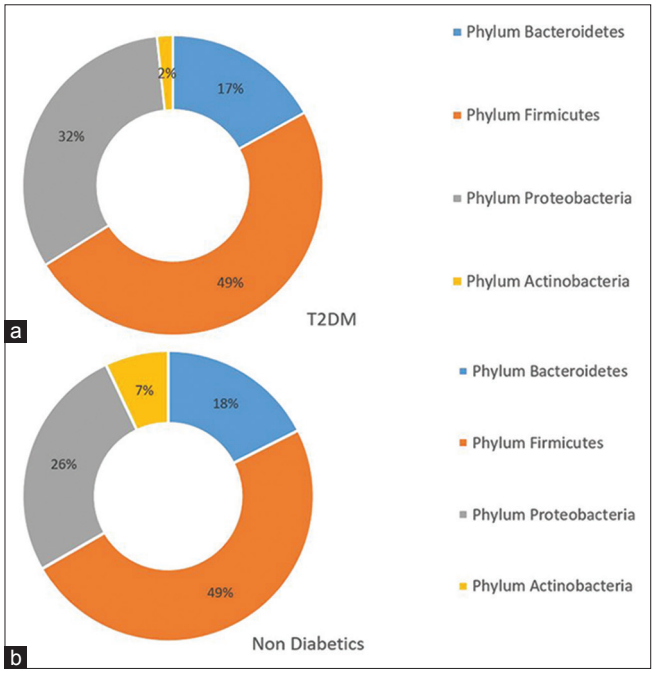

Figure 1: (a) Phyla encountered in type 2 diabetes mellitus, (b) phyla encountered in nondiabetics

We then stratified the gut microbiota based on HbA1c as 6.5–7.5%, 7.6–8.5% and > 8.5%. Analysis of genera based on HbA1c showed that Gram-negative bacteria were predominant among T2DM with HbA1c between 6.5%–7.5% and 7.6%–8.5% [Figure 2].

Chi-square test has shown a statistical significance for the genera *Escherichia* among T2DM and *Bifidobacteria* among nondiabetics.

We also applied principal component analysis (PCA) using R software (version 3.0.2) to observe the intercorrelation among bacterial genera across diabetics and nondiabetics. The biplot of 1st PCA (27% variation explained) and 2nd PCA (18% variation explained) generated is shown in Figure 3a and b. The biplot indicates that *Faecalibacterium* and *Bifidobacterium* are very strongly associated and form a cluster along with *Eubacterium*. On the other hand,

Table 4: Profile of different genera among type 2 diabetes mellitus and nondiabetics with percentage and counts of predominant bacteria

		T2DM (*n*=17)			Nondiabetic (*n*=13)			
Phylum	**Genera**	**Percentage**	**Counts of bacteria**	**BMI mean**	**Genera**	**Percentage**	**Counts of bacteria**	**BMI mean**
Proteobacteria	*Escherichia*	58.8*	96,466	30.4	*Escherichia*	7.7*	109,734	27.7
		41.2+	5739	29.9		92.3+	2593	25.7
Firmicutes	*Faecalibacterium*	47.1*	58,213	29.7	*Faecalibacterium*	61.5*	67,935	24.9
		52.9+	878	30.7		38.5+	7191	27.4
	Lactobacillus	35.3*	10,217	29.9	*Lactobacillus*	7.7*	3847	32.9
		64.7+	6343	30.4		92.3+	10,702	25.3
	Clostridium	23.5*	17,537	31.9	*Clostridium*	30.8*	14,340	26.1
		76.47+	20,160	29.7		69.23+	23,684	25.8
	Eubacterium	23.5*	17,752	30.2	*Eubacterium*	46.2*	42,704	27.6
		76.5+	6752	30.2		46.2+	4422	24.4
	Ruminococcus	23.5*	6998	27.2	*Ruminococcus*	7.7*	1400	32.9
		76.47+	3027	31.2		92.3+	9806	25.3
Bacteroidetes	*Bacteroides*	11.8*	1614	28.3	*Bacteroides*	15.4*	10,061	24.6
		88.23+	5303	30.5		84.6+	6075	26.1
	Prevotella	47.1*	48,943	29.4	*Prevotella*	23.1*	12,121	25.6
		52.9+	1336	30.9		76.9+	13,132	25.9
Actinobacteria	*Bifidobacteria*	5.9*	16,183	31.6	*Bifidobacteria*	38.5*	29,803	25.7
		94.1+	2659	30.1		61.5+	2707	25.9

T2DM: Type 2 diabetes mellitus, BMI: Body mass index, *: Percentage of bacteria present, +: Percentage of bacteria absent

Lactobacillus and *Prevotella* were found to be closer in the biplot, hence it is expected to be associated with each other, but *Escherichia* and *Bacteroides* are found to be completely independent. Furthermore, *Faecalibacterium*, *Eubacterium,* and *Bifidobacterium* have very strong contribution to 1st PCA, whereas *Lactobacillus*, *Prevotella*, and *Eubacterium* have very strong contribution to 2nd PCA [Figure 3a]. PCA has also confirmed that *Escherichia* is predominant genera among diabetic subjects [Figure 3b].

We observed that as the HbA1c increased, the counts of *Escherichia* decreased, but that was not statistically significant. A difference was however observed which is shown in Figure 4a with median and interquartile range using Kruskal–Wallis test.

Table 5: Correlation of *Escherichia* and *Bifidobacteria* among type 2 diabetes mellitus and nondiabetics

Bacteria	T2DM (%)	Nondiabetics (%)	*P*
Escherichia+	10 (58.8)	1 (7.7)	0.007
Escherichia−	7 (41.2)	12 (92.3)	
Bifidobacteria+	1 (5.9)	5 (38.5)	0.04
Bifidobacteria−	16 (94.1)	8 (61.5)	

T2DM: Type 2 diabetes mellitus

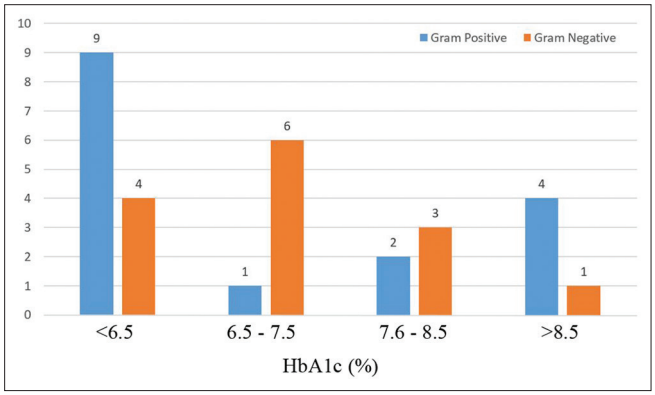

Figure 2: Gram positive versus Gram negative genera profile of gut microbiota among nondiabetics (<6.5) and type 2 diabetes mellitus (based on glycated haemoglobin stratification)

Escherichia count was higher in the obese than nonobese subjects in the T2DM group, but this was not statistically significant (Wilcoxon rank sum test) [Figure 4b].

Analysis of MCP-1 [Figure 5] in T2DM subjects with HbA1c of 6.5–7.5% showed a mean of 170.08 pg/ml with a range of 56.3–319.3 pg/ml in subjects with HbA1c of 7.6–8.5% and > 8.5%. MCP-1 values ranged from 87.4 to 140.9 pg/ml and 155-241.2 pg/ml with a mean of 115.2 and 198.35 pg/ml, respectively. In the nondiabetics, MCP-1 value ranged from 66.9 to 236.8 pg/ml with a mean of 132.3 pg/ml. Although MCP-1 values were higher in both HbA1c groups, it was not statistically significant ($P = 0.16$ and 0.07, respectively).

We then compared IFNγ with HbA1c and found that IFNγ value, mean, and range of 385.5 pg/ml and 414.8–984.4 were higher in T2DM group of 6.5–7.5% which was statistically significant using Student's *t*-test ($P = 0.013$). IFNγ values across the other two HbA1c groups and nondiabetics were below detectable level [Figure 6].

Discussion

Gut microbiota and their role in obesity and T2DM are being extensively studied.[2,12] There have been attempts to develop identity marker for gut microbiota among subjects with T2DM or other metabolic disorders. However, the role of gut microbiota in T2DM, especially in relation to HbA1c, has not been well-studied. This is a preliminary study conducted using fecal samples obtained from subjects with HbA1c ranging from 6.5 to > 8.5% and nondiabetics with HbA1c of < 6.5%. The gut microbial composition

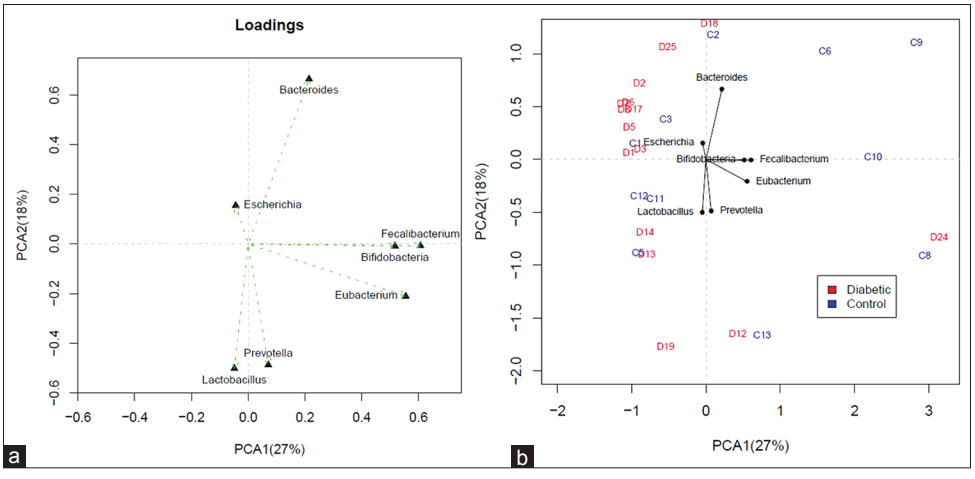

Figure 3: (a) Relationship between the genera of gut microbiota among type 2 diabetes mellitus. (b) clustering of type 2 diabetes mellitus along with the predominant genera in type 2 diabetes mellitus

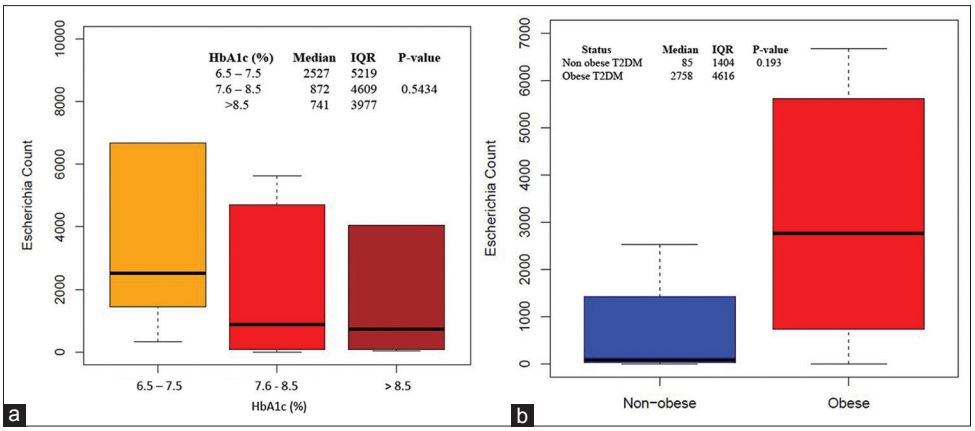

Figure 4: (a) Relationship between *Escherichia* and glycated hemoglobin. (b) Relationship between *Escherichia* and body mass index

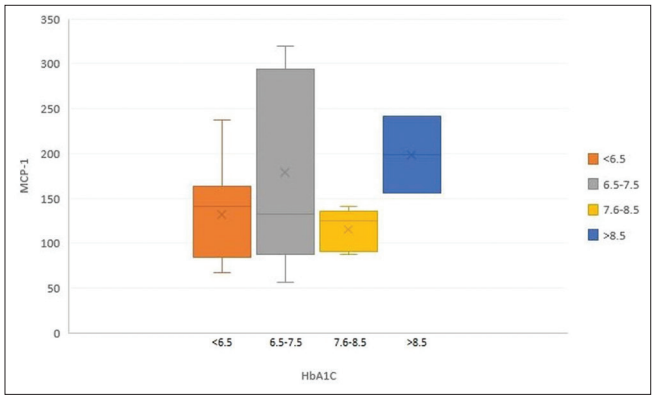

Figure 5: Range of monocyte chemoattractant protein-1 in relation to glycated hemoglobin in type 2 diabetes mellitus and nondiabetics

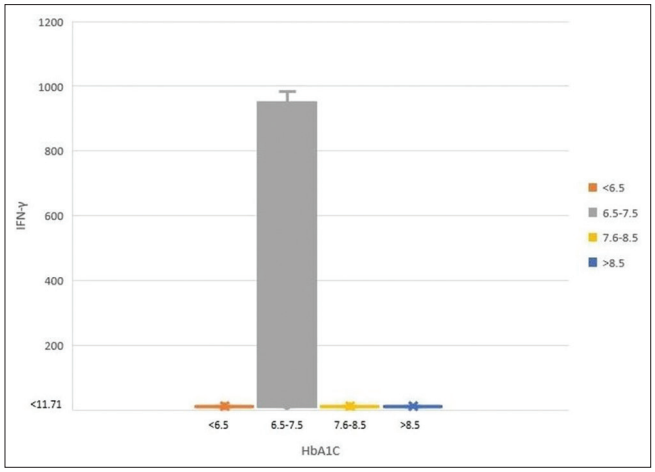

Figure 6: Range of interferon gamma in relation to glycated hemoglobin in type 2 diabetes mellitus and nondiabetic

of the subjects was studied by metagenomic sequencing targeting the V6 region of the 16S rRNA gene. The gut microbiota has also been correlated with BMI and inflammatory markers such as MCP-1 and IFNγ.

No significant difference in bacterial phyla was observed in T2DM and nondiabetics. Firmicutes predominated in both groups 50% T2DM and 53% nondiabetics followed by Proteobacteria (33 and 28%) and Bacteroidetes (17 and 19%), respectively.

However, the presence of Proteobacteria is different from previous reports. Phylum Proteobacteria was slightly abundant in T2DM than in nondiabetics, and this is a potential diagnostic marker of dysbiosis and risk of disease.[13]

Escherichia was predominant in T2DM patients and positively correlated with HbA1c and BMI. Enterobacteriaceae are reported to be a major cause of mortality and morbidity in T2DM.[14] In addition, a threefold increased risk of complications due to *Escherichia* and other Enterobacteriaceae has been observed.[15] Furthermore, intake of artificial sweeteners impairs the glucose control and can elevate the Proteobacteria levels.[16,17]

Certain species of *Lactobacillus* may contribute to chronic inflammation in T2DM. We observed 35.3% subjects of T2DM had *Lactobacillus*. Several strains of *Lactobacillus* are administered as probiotics, for example, *Lactobacillus paracasei* ssp. paracasei F19 and *Lactobacillus gasseri* SBT2055 are known to reduce visceral and subcutaneous fat. Certain species may contribute to inflammation. A limitation of the study was that we were unable to speciate *Lactobacillus*.

Bifidobacterium, a dominant member of the intestinal microbiota and probiotic strain of the phylum Actinobacteria, was increased in the nondiabetics than in T2DM. It has been reported that endotoxaemia negatively correlates with *Bifidobacterium* and positively correlates with improved glucose tolerance, glucose-induced insulin secretion, decreased endotoxemia, and adipose tissue proinflammatory cytokines.[18] This is because *Bifidobacterium* improves mucosal barrier function thereby decreasing the endotoxin levels.[19,20]

In our study, Gram-negative bacteria were found to be higher than Gram-positive bacteria in T2DM when compared with nondiabetics. Lipopolysaccharide (LPS) is a major component of the Gram-negative cell wall, and the human gut serves as a reservoir of LPS. LPS levels were low in healthy subjects suggesting that it is absorbed at a low rate in the gut. Increased level of Gram-negative gut bacteria correlates with high circulating LPS and high insulin resistance in T2DM.[21] Studies have shown that high fat feeding augments LPS concentrations sufficient to increase body weight, fasting glycemia, and inflammation. Further, LPS was identified as a triggering factor in the early development of metabolic diseases and is also a strong stimulator of several cytokines that cause insulin resistance and metabolic endotoxemia.[21] Normally, the intestinal epithelium acts as a continuous barrier to avoid LPS translocation; yet some endogenous or exogenous events may alter this protective function.

When we analyzed the correlation of gut microbiota with BMI alone, we found that Proteobacteria was increased in obese subjects and absent in subjects with normal BMI among T2DM. *Escherichia* positively correlated with obese T2DM subjects.

In our study, we found that MCP-1 was elevated across HbA1c of 6.5–7.5% and > 8.5% when compared with nondiabetics. We have previously reported MCP-1increases by 37.3 pg/ml for every 1% increase in HbA1c among patients with periodontal disease and T2DM.[22]

IFNγ is another proinflammatory cytokine which plays a crucial role in inflammatory disease.[23] The gluco-toxic microenvironment created in diabetes may influence the cell to cell signaling capabilities of cytokines.[24] IFNγ may contribute to complications occurring in T2DM and also play a protective role in healthy subjects.[24] Furthermore, increased IFNγ concentrations delay the development of microvascular complications in T2DM.[24] Al-Shukaili *et al*. reported an increase in IFNγ in T2DM patients than normal patients.[25] In our study, IFNγ levels were significantly increased in T2DM subjects with HbA1c of 6.5–7.5% indicating that it rises early in the disease-causing inflammation.

The composition of gut microbiota in our study subjects differed from those previously reported in the West. These differences may be due to the influence of diet and need to be studied prospectively.

We found similar genera between three subjects, two of whom were nondiabetic and one T2DM. All the subjects were unrelated to each other and came from different background. Twins and their families have reported an identical gut microbiota.[26] In our study since all were unrelated, probably similarities in diet may have resulted in an identical gut microbiota.

To the best of our knowledge, this is the first study to profile gut microbiota in T2DM subjects and to compare them with nondiabetics as well as correlate with MCP-1 and IFNγ.

Conclusion

Escherichia counts were elevated in T2DM with HbA1c of 6.5-8.5% which was statistically significant suggesting that lipopolysaccharides present in the cell wall of Gram-negative bacteria may be responsible for low-grade inflammation as evidenced by elevated MCP-1 and IFNγ levels in T2DM with the same HbA1c levels. The gut microbiota of nondiabetics is characterized by predominance of *Bifidobacteria* and other Gram-positive bacteria.

Financial support and sponsorship

The authors greatly acknowledge Research Society for the Study of Diabetes in India (RSSDI) for funding the study.

Conflicts of interest

There are no conflicts of interest.

References

1. Murea M, Ma L, Freedman BI. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Rev Diabet Stud 2012;9:6-22.
2. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 2010;5:e9085.
3. Available from: http://www.idf.org/diabetesatlas. [Last update on 2014].
4. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/. [Last accessed on 2015 Dec 17].
5. Available from: http://www.idf.org/sites/default/files/DA regional-factsheets-2014_FINAL.pdf. [Last accessed on 2015 Dec 17].
6. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006;124:837-48.
7. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005;102:11070-5.
8. DiBaise JK, Frank DN, Mathur R. Impact of the gut microbiota on the development of obesity: Current concepts. Am J Gastroenterol Suppl 2012;1:22-7.
9. Mikkelsen KH, Frost M, Bahl MI, Licht TR, Jensen US, Rosenberg J, *et al.* Effect of antibiotics on gut microbiota, gut hormones and Glucose Metabolism. PLoS One 2015;10:e0142352.
10. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: The hygiene hypothesis expanded? Diabetes Care

2010;33:2277-84.
11. Andrew G. Gut microbiota, low-grade inflammation, and metabolic syndrome. FASEB J 2015;29 1 Suppl: 368-2.
12. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, *et al*. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013;498:99-103.
13. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol 2015;33:496-503.
14. Graff LR, Franklin KK, Witt L, Cohen N, Jacobs RA, Tompkins L, *et al*. Antimicrobial therapy of gram-negative bacteremia at two university-affiliated medical centers. Am J Med 2002;112:204-11.
15. Thomsen RW, Hundborg HH, Lervang HH, Johnsen SP, Schønheyder HC, Sørensen HT. Diabetes mellitus as a risk and prognostic factor for community-acquired bacteremia due to enterobacteria: A 10-year, population-based study among adults. Clin Infect Dis 2005;40:628-31.
16. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, *et al*. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 2014;514:181-6.
17. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, *et al*. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 2015;519:92-6.
18. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, *et al*. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50:2374-83.
19. Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, *et al*. *In vivo* effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. Dig Dis Sci 2004;49:579-89.
20. Wang Z, Xiao G, Yao Y, Guo S, Lu K, Sheng Z. The role of bifidobacteria in gut barrier function after thermal injury in rats. J Trauma 2006;61:650-7.
21. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761-72.
22. Radhakrishnan P, Srikanth P, Seshadri KG, Barani R, Samanta M. Serum monocyte chemoattractant protein-1 is a biomarker in patients with diabetes and periodontitis. Indian J Endocrinol Metab 2014;18:505-10.
23. Chang SH, Chung Y, Dong C. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. J Biol Chem 2010;285:38751-5.
24. Alfred A, Anthonia OO, Chukwuma JO, Donatus FNO. Interplay of T Helper 1 and 2 Cytokines in Type 2 Diabetes Mellitus With and Without Microvascular Complications. Int J Biol Med Res 2011;2: 917-21.
25. Al-Shukaili A, Al-Ghafri S, Al-Marhoobi S, Al-Abri S, Al-Lawati J, Al-Maskari M. Analysis of inflammatory mediators in type 2 diabetes patients. Int J Endocrinol 2013;2013:976810.
26. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, *et al*. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.