



Gut Microbe Analysis between Asthma Patients and Healthy Volunteers in Shaanxi Province, Xi'an, China

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ABSTRACT

Asthma is an allergic and chronic lung disease that causes inflammation and narrows the air passages. Present study aimed to characterize the gut microbiota of asthma patients and to investigate the alteration in similarity and diversity of gut microbial composition with comparison of healthy controls. Fecal samples from fifteen patients and five healthy individuals were collected. PCR-DGGE, by using universal primers focusing V3 region of 16S rRNA gene, was performed for characterization of gut microbial composition. Sequencing of most dominant excised gel bands was done. Real-time PCR was performed to evaluate the copy numbers of dominant bacteria of gut microbiota. The results indicated that a significant diversity difference between asthma and healthy groups ($*P < 0.05$), was shown in DGGE profile. Similarity index was found to be lower in inter-group than intra-group that indicated a change in composition of gut flora in asthma patients. Sequencing results also indicated a change of bacterial composition between both groups. qPCR results showed a significant decrease in *Bifidobacterium*, *Lactobacillus* and *Clostridium leptum* sub group ($*P < 0.05$) and non-significant decrease and increase in *Bacteroides vulgatus* and *Peptostreptococcus productus*, respectively. Summarizing all, in asthma patients, there is a significant change in the molecular characterization of gut microbiota.

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Authors' Contribution

HMI performed all the experiment and prepared initial draft of the manuscript. MS revised the manuscript, XW guided to collect the samples for this study and CM helped to collect the samples. JX supervised the research project and provide guidance and support to do research.

Key words

Asthma, Gut microbiota, Real Time PCR, DGGE profile.

INTRODUCTION

Human gut microbiota is an important factor in determining the health status of the host. Its composition and functional activities are stable over time but may be altered by different factors including age, disease and diet (Power *et al.*, 2013). Human gut flora contains approximately 100 trillion bacteria and plays a vital role in human physiology including metabolism, nutrition absorption and immune function (Walsh *et al.*, 2014; Ishaq *et al.*, 2017a). Gut microbiota plays an important role in host immune homeostasis and produces defense against pathogens (Kamada *et al.*, 2013; Tan *et al.*, 2013).

Modulation of gut microbial composition has been associated with many other diseases *i.e.* colitis, Crohn's disease, hyperthyroidism, viral diarrhea, inflammatory bowel disease, metabolic diseases such as obesity, and

type II diabetes (Ma *et al.*, 2011; Zhou *et al.*, 2014; Khoruts *et al.*, 2010; Ishaq *et al.*, 2017b).

Asthma is an allergic chronic inflammatory disease that causes a substantial burden on patients and their families (Bonamichi-Santos *et al.*, 2015). Over the last decade, asthma prevalence has dramatically increased in Western societies. It is a complex disease having strong genetic and environmental components that manifests with a variety of clinical features *i.e.* short breathing, cough, chest congestion, wheezing and sputum production (Huang and Boushey, 2015). A dysbiotic gut flora may have an important role for developing allergic diseases (Muir *et al.*, 2016).

Denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rRNA genes has been performed to evaluate the gut microbial diversity (Wang *et al.*, 2016). It gives a DNA fingerprinting of experimental samples; also provides the help to identify the bacterial community through sequence analysis and to check their dynamics. For the characterization of complex bacterial communities, qPCR and 16S rRNA gene libraries are marvelous

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development (Dorigo *et al.*, 2005).

PCR–DGGE along with image analysis helps to study the microbial similarity and diversity, while erection of dendrogram (UPGMA) and analysis of sequence were done to study the disease linked DGGE motifs and taxa (Wu *et al.*, 2010). Real-time PCR was performed by using primers of *Clostridium leptum* sub group, *Bacteroides vulgatus*, *Peptostreptococcus productus*, *Lactobacillus* and *Bifidobacterium* genera for quantitative evaluation of gut microbiota of asthma patients.

The aim of current study was the characterization of gut microbiota of asthma patients and to investigate and study the alteration in similarity and diversity of gut microbial composition in comparison with healthy controls.

MATERIAL AND METHODS

Sample collection and ethics statement

A detailed semi-structured questionnaire was given to each volunteer after they had received and read a complete description of the study. All participants provided written informed consent and all the protocols were approved by the Ethics Committee of School of Medicine, Xi'an Jiaotong University. Additionally, the research and recruitment protocols were carried out according to the Ethical Principles for Medical Research Involving Human Subjects adopted in the Declaration of Helsinki by the World Medical Association. All samples used in the study followed standardized rules governing sample handling, and information obtained in the interview was recorded on standardized data collection forms.

Fecal samples were collected from 15 asthma (7 female and 8 males, having age between 30 to 45 years) and 5 healthy volunteers (2 females and 3 males, having same age between 30 to 45 years) in a sterile cup. A questionnaire was filled regarding age, gender, dietary habits, body weight, and health status of patients as well as healthy volunteers. Two patients were tracked for about three weeks to check the stability of gut microbiota (samples collected on day 1, 7, 14 and 21 days). All the samples were delivered on ice, usually within 4 hour of defecation. Upon arrival in the laboratory, fecal sample were stored at -80 °C until DNA extraction. None of the patients and healthy individuals had any record of gastrointestinal disease; also, there was no history of using prebiotics, probiotics and antibiotics, two months prior to sampling.

DNA extraction from fecal sample

After thawing of all fecal samples, DNA was extracted with the help of QIAGEN QIAamp Stool kit (QIAGEN, Germany) as per instructions of manufacturer,

with initial start of bead-beating step at 5000 rpm for 30 sec. Concentration of DNA was evaluated by the use of NanoPhotometer TM (IMPLEN, Germany) (Scanlan *et al.*, 2006).

PCR amplification for DGGE

Fecal bacterial DNA was used for PCR–DGGE. Universal primers (Table I) were used to amplify the 16S rRNA gene (V3 region). 50 µl PCR reaction mixture contain 20 pmol of two primers, 2.5 mM 10×buffer, MgCl₂ 2.5 mM, Taq (Promega, USA) DNA polymerase 2 U, deoxy nucleotide triphosphate (dNTP) 200 mM and 120 ng DNA approximately 2 µl. An automatic thermal cycler (ABI2720 made in USA) was used to amplify the PCR mixture through PCR touchdown programming: initial start with denaturation of PCR mixture at 95 °C for 5 min, then denature by 95 °C for 1 min, annealing temperature reduced to 65 °C for 1 min and then to increase 72 °C for 1 min. Decrease of annealing temperature 1°C in every second cycle so that touchdown temperature become 55 °C, at this stage of temperature, 10 extra cycles were carried out, and then followed by 72 °C for 7 min for final extension and finally maintained at 4 °C. 1.5% agarose gel were used to electrophoresis the amplified PCR mixture and dipped in ethidium bromide solution to visualize under UV light (Muyzer *et al.*, 1993).

Table I.- Primers used in PCR-DGGE.

Primer	Sequence (5'–3')
341-F	CCTACGGGAGGCAGCAG
534-R	ATT ACCGCGGCTGCTGG
341FG	CGCCCGCCGCGCGCGGGCGG GCGGGGCGGGGGCACGGG GGCCTACGGGAGG CAG CAG

Reference: Matsuki *et al.* (2004).

Denaturing gradient gel electrophoresis

In brief, amplified PCR product of total bacteria was loaded in 8% (w/v), acrylamide-bis, 37.5:1, gels in a 1×TAE buffer solution tank, having 30–65 % linear denaturant gradient. The gel was run for 13 h at 90 V with constant temperature at 60°C. Calculation of bacterial diversity of each sample was done by the total number and the strength of DGGE bands, with the help of Quantity one software. A similarity index was studied through Dice's similarity coefficient (van Der Gucht *et al.*, 2001). UPGMA (arithmetic averages) through unweighted pair group method, was used to establish a dendrogram (Ling *et al.*, 2010).

Statistical analysis of DGGE band pattern

The number of bands and band intensity of DGGE profiles was determined by using Quantity one software.

Taxonomic diversity of bacteria was evaluated by Shannon–Weaver index of diversity (H^1) (Gafan *et al.*, 2005; Ledder *et al.*, 2007). Similarity index and DGGE profile's cluster analysis were done through UPGMA method (band based Dice similarity coefficient). As there was no uniformity in data distribution, Mann–Whitney U test was used for a nonparametric statistical analysis, where ($P < 0.05$) was considered as statistically significant. Arithmetic averages (UPGMA) and clustering algorithm were used to calculate the dendrograms for unweighted pair group method (Fromin *et al.*, 2002; Silva and Russo, 2000). To observe the stability of gut microbial composition, two patients were checked for a period of 3 weeks; gut microbial DGGE band analysis showed high similarity in bands patterns. Dice similarity coefficient ranged between 96–97% (Figure not shown).

Calculation of Shannon–Weaver index (H^1) was done with the help of following equation:

$$\text{Shannon – Weaver index } (H^1) = - \sum_{i=1}^s (P_i)(\ln P_i)$$

Excision of bands and sequencing

Physically a sterilized scalpel was used to excise the dominant band of interest from the gel with care. The polyacrylamide gel piece was placed in a tube containing 50 μ l of water and incubated at 37 $^{\circ}$ C for 30 min. After centrifugation, 8 μ l of DNA template was used to re-amplify the V3 region of 16S rRNA gene by using original primers (without GC clamp) as previously used for DGGE analysis (Green *et al.*, 2010). Sequencing of re-amplified PCR products were done by ABI 3500xL. Obtained Sequences were analyzed by using BLAST software for identification of species or genus (McBain *et al.*, 2003).

Real-time PCR

Real time PCR quantification was performed in a

CFX96 Bio-Rad (USA). Total 20 μ l reaction mixture contained 1 μ l of each primer (5 μ M), 10 μ l of 2 \times SYBR Green (TOYOBO, Japan), 2 μ l sample DNA and 6 μ l sterilized H₂O. Amplification program composed of 1 cycle at 95 $^{\circ}$ C for 5 min, then followed by 40 cycles at 95 $^{\circ}$ C for 10 sec. then 55 $^{\circ}$ C for 15 sec. and followed by 72 $^{\circ}$ C for 50 sec. and finally 1 cycle at 95 $^{\circ}$ C for 15 sec. The fluorescent signals were detected at the end of every cycle. After amplification, PCR melting temperature analysis was done to evaluate the specificity of the PCR system. Through slow heating at 0.1 $^{\circ}$ C/s with increase from 65 $^{\circ}$ C to 95 $^{\circ}$ C, melting curves were achieved, with continuous fluorescence deduction. Real time PCR Primers were shown in Table II. To determine the copy number of *Clostridium leptum* sub group, *Peptostreptococcus productus*, *Bacteroides vulgatus*, *Lactobacillus* and *Bifidobacterium* genus in each DNA samples, in same experiment, fluorescent signals were calculated, averaged and compared with standard curve that were generated with standard DNA from six serial dilutions in linear range of the program (Ma *et al.*, 2011; Wu *et al.*, 2010). *Bacteroides vulgatus* (CICC 22938), *Bifidobacterium* (CICC 6186), NWS *Lactobacillus*, *Peptostreptococcus productus* (from our lab) and *Clostridium leptum* sub group (YIT 6169) were used as standard strains. Real time PCR was done thrice and mean was considered in results. Data were reported as the average estimate of logarithms of fecal PCR target genetic amplicon copy numbers present in 1g of feces.

Statistical analysis

Real time PCR and PCR-DGGE experiments were repeated thrice. Statistics software SPSS 12 was applied for statistical analysis. P values were calculated by using the U (Mann-Whitney) test. Results were considered as statistically significant, where ($P < 0.05$).

Table II.- Primers that were used in real time PCR.

Target bacteria		Primer Sequence (5'–3')	Band size
<i>Bifidobacterium</i>	Bifid F	CTC CTGGAAACGGGTGG	550 bp
	Bifi-R	GGTGTCTTCCCGATATCTACA	
<i>Lactobacillus</i>	LactF	CTC AAA ACT AAACAAAGTTTC	250 bp
	LactR	CTC AAA ACT AAACAAAGTTTC	
<i>Bacteroides vulgatus</i>	BV- F	GCATCATGAGTCCGCATGTTC	287 bp
	BV-R	TCC ATA CCC GACTTT ATT CCTT	
<i>Clostridium leptum</i>	C.lep-F	GCACAAGCAGTG GAG T	239 bp
	C.lep-R	CTTCTCCGTTTTGTCAA	
<i>Peptostreptococcus productus</i>	PSP-F	AACTCCGGTGGTATC AGA TG	268 bp
	PSP-F	GGGGCTTCT GAG TCAGGTA	

Reference: Matsuki *et al.* (2002), Dubernet *et al.* (2002) and Wang *et al.* (1996).

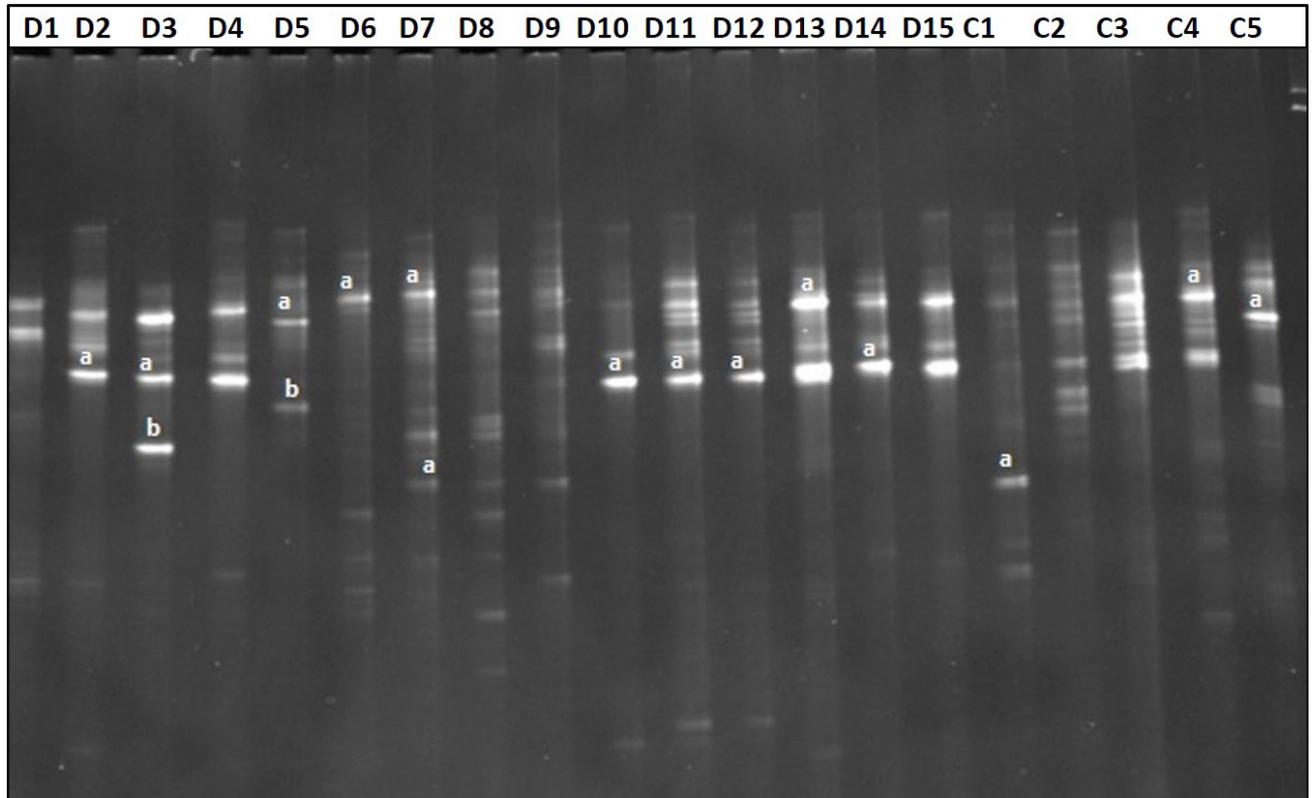


Fig. 1. DGGE gel profile through deploying of universal linkage primer of 16S rRNA gene with pointing V3 region of asthma patients group (D1-D15) and healthy control group (C1-C5).

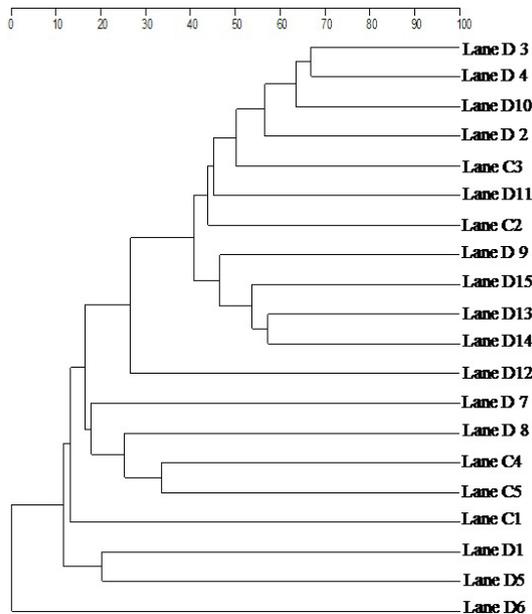


Fig. 2. Cluster analysis between asthma patients (D1-D15) and healthy control (C1-C5) by applying UPGMA and Dice's similarity coefficient.

RESULTS

DGGE profiles of asthma and healthy group

DGGE analysis was done with amplified PCR product by using universal primers targeting the 16S rRNA (V3 region) gene in both healthy and asthma group. In Figure 1, D1–D15 indicate samples from asthma and C1–C5 healthy controls. As the position, number, and bands intensity were different among experimental samples, which showed the complex intestinal microbial fingerprints. In DGGE gel, there were some common bands and some were less intense bands present in different samples, at the same position but in different lane. A total 74 bands were detected by using Quantity one software (van Der Gucht *et al.*, 2001), in 15 tracks of asthma with average band of (5.7 ± 2.015) . A total 34 bands were detected in 5 tracks of control group with average of (6.8 ± 1.095) . For analysis of gut microbial composition in asthma and healthy group, *U* (Mann-Whitney) test was applied for comparison of Shannon weaver index (H') of diversity. The diversity (H') results indicated $(2.00 \pm 0.31$ vs. $2.27 \pm 0.21)$ significant ($P < 0.046^*$) difference between the two groups. Similarity index results showed

to be lower in inter-group than intra-group that indicated a change in composition of gut flora in asthma patients. DGGE profiles similarity was determined through Dice similarity coefficient and dendrogram UPGMA shown in Figure 2. The band-based Dice similarity coefficient values of asthma and healthy group and with the mean similarity index were (0.235 ± 0.200) and (0.219 ± 0.151) respectively, shown in Table III. When all the values of statistical samples of asthma and the healthy group were compared by Dice similarity coefficient, mean similarity index was (0.205 ± 0.127) between two groups, which showed that it was to be lower in inter-group than intra-group, which shows that gut microbial composition of asthma patients was different from healthy control group.

Sequencing results analysis of dominant band

In Figure 1, 16 bands from DGGE gel were excised for quantity analysis. To certify the resolution capability

of DGGE bands in different lane but same positions, the bands C4a and D13a, D10a, D11a and D12a were cut for sequence analysis. Bands C4a and D13a were identified as *Bacteroides vulgatus* with 95 % similarity while, bands D10a, D11a and D12a were identified as *Klebsiella oxytoca* with 94 % similarity. Taxonomic identity of other bands has been shown in Table IV. Sequencing results were analyzed by using BLAST. Results indicated that phyla Firmicutes, Bacteroidetes, and Proteobacteria were dominant.

Real time PCR quantification

The *Bifidobacterium*, *Peptostreptococcus productus*, *Bacteroides vulgatus*, *Clostridium leptum* and *Lactobacillus* were quantified by real time PCR. Results showed that the copy number of *Bifidobacterium* (6.10 ± 0.25 vs. 6.54 ± 0.67), *Lactobacillus* (7.61 ± 1.05 vs. 8.65 ± 0.09) and *Clostridium leptum* sub group (4.32 ± 0.28 vs.

Table III.- Gut microbial similarity and diversity of asthma patients and healthy subjects.

Groups	Diversity		Similarity	
	Number of bands ^a	Shannon index ^b	Intra-similarity ^c	Inter-similarity ^d
Disease group	5.7 ± 2.015	2.00 ± 0.31	0.235 ± 0.200	0.205 ± 0.127
Control group	6.8 ± 1.095	2.27 ± 0.21	0.219 ± 0.151	
P Value	0.132	0.046*	-	-

Results that are significantly different with $P < 0.05$ (Mann–Whitney *U* test); ^aBands numbers, denaturing gel electrophoresis (DGGE) produced by each sample; ^bShannon diversity index (*H'*) was calculated by using the relative intensities of all DGGE bands present in each sample; ^cComparing of DGGE band profiles within individual of a given groups by Dice similarity coefficients; ^dComparing of DGGE band profiles between members of asthma and healthy groups by Dice similarity coefficients.

Table IV.- Sequences of excised PCR Amplicons from DGGE gel and identities based by BLAST database.

Selected excised bands	Bacteria with highest % homology	Sequence accession number	Bacterial phyla	Gene bank number
D2a	<i>Sporomusa ovate</i> (89)	DSM 2662	Fermicutes	NZ_ASXP01000005.1
D3a	<i>Escherichia coli</i> (98)	MG1655	Protobacteria	NC_000913.3
D3b	<i>Bacteroides uniformis</i> (98)	CL03T00C23	Bacteroidetes	NZ_JH724260.1
D5a	<i>Alistipes putredinis</i> (89)	DSM 17216	Bacteroidetes	NZ_DS499580.1
D5b	<i>Ruminococcus flavefaciens</i> (87)	MC2020	Fermicutes	NZ_JNKE01000007.1
D6a	<i>Prevotella copri</i> (95)	CB7	Bacteroidetes	NR_040877.1
D7a	<i>Bacillus pumilus</i> (87)	NJ-M2	Fermicutes	NZ_CP012329.1
D7b	<i>Bacteroides pyogenes</i> (90)	JCM 10003	Bacteroidetes	NZ_BAIU01000058.1
D10a	<i>Klebsiella oxytoca</i> (94)	CAV1374	Protobacteria	NZ_CP011636.1
D11a	<i>Klebsiella oxytoca</i> (94)	CAV1374	Protobacteria	NZ_CP011636.1
D12a	<i>Klebsiella oxytoca</i> (94)	CAV1374	Protobacteria	NZ_CP011636.1
D13a	<i>Bacteroides vulgatus</i> (95)	ATCC 8482	Bacteroidetes	NC_009614.1
D14a	<i>Parabacteroides distasonis</i> (90)	ATCC 8503	Bacteroidetes	NZ_BAIU01000058.1
C1a	<i>Prevotella histicola</i> (90)	F0411	Bacteroidetes	NZ_JH376764.1
C4a	<i>Bacteroides vulgatus</i> (95)	ATCC 8482	Bacteroidetes	NC_009614.1
C5b	<i>Bacteroides coprocola</i> (97)	DSM 17136	Bacteroidetes	NZ_DS981502.1

Table V.- Real time PCR quantification (Mean \pm SD) of different bacteria.

Bacteria	Healthy subjects	Patients	P value
<i>Bifidobacterium</i> (10^5)	6.54 \pm 0.67	6.10 \pm 0.25	0.020*
<i>Bacteroides vulgatus</i> (10^8)	2.30 \pm 1.51	1.90 \pm 1.05	0.319
<i>Lactobacillus</i> (10^6)	8.65 \pm 0.09	7.61 \pm 1.05	0.021*
<i>Clostridium leptum</i> (10^6)	4.59 \pm 0.19	4.32 \pm 0.28	0.031*
<i>Peptostreptococcus productus</i> (10^2)	1.40 \pm 1.00	1.80 \pm 0.90	0.334

Results were reported through the average estimate of logarithms in fecal PCR target genetic amplicon, copy numbers present in 1 g of feces* indicates (P<0.05).

4.59 \pm 0.19) were significantly (*P<0.05) decreased in the patients as compared to healthy subjects. While the copy number of *Peptostreptococcus productus* (1.80 \pm 0.90 vs. 1.40 \pm 1.00) and *Bacteroides vulgatus* (1.90 \pm 1.05 vs. 2.30 \pm 1.51) were non-significantly (P<0.05) increased, and decreased respectively, in the fecal samples of patients as compared to healthy subjects. All these results are summarized in Table V.

DISCUSSION

Human gut microbiota plays a vital role to protect the body against diseases by performing metabolic, trophic and protective function (Guarner and Malagelada, 2003). Gut microbial composition can be altered in many diseases like Crohn's disease, malnutrition, inflammatory bowel disease, colitis, hyperthyroidism, obesity and type II diabetes (Ma *et al.*, 2011; Zhou *et al.*, 2014; Khoruts *et al.*, 2010). Our study showed that there was difference between gut microbial composition of asthma patients and healthy subjects as determined by sequence of dominant bands from DGGE profile and real time PCR analysis. More precisely, there were significant differences in the diversity and similarity of bacterial communities, measured by Shannon-weaver index (H') and Dice similarity coefficient. The bacterial copy number of *Bifidobacterium*, *Lactobacillus* and *Clostridium leptum* sub group were significantly decreased in asthma patients group as compared to control group. While *Peptostreptococcus productus* and *Bacteroides vulgatus* were non-significantly increased, and decreased in diseased group as compare to healthy controls, respectively. In current study, in asthma patients group and healthy control group, bacterial similarity and diversity of the gut microbiota were analyzed by combining DGGE profile (targeting 16S rRNA gene) with imaging and sequencing of dominant PCR amplicons, together with statistical analyses. DGGE fingerprint techniques focusing 16S rRNA gene and quantification of

major bacteria with real time PCR have been used to study the complex bacterial communities (Zhou *et al.*, 2014; Ma *et al.*, 2011). To evaluate the diversity of gut microbial composition, in asthma and control group, we calculated the Shannon–Weaver index (H'). Compared to healthy individuals, (H') was significantly lowered in asthma group (P < 0.046*). The decreased bacterial diversity might have altered the gut microbial composition in asthma. While similarity index was found to be lower in inter-group than intra-group, which showed that gut microbial composition was changed in asthma patients group. In our study, gut microbial diversity, Shannon–Weaver index (H'), results are consistent with previous study of gut microbe analysis of hyperthyroid patients (Zhou *et al.*, 2014). It shows that gut microbial composition of patients varies to different degree as a result of asthma occurrence. Hence, asthma may seriously influence the composition of gut microbiota. These results indicate that due to occurrence of asthma, it may cause the physiological intestinal changes which may result in alteration of gut microbial composition. Furthermore, the change in gut microbial composition may lead to the worsening of sickness (Kamada *et al.*, 2013).

Previous studies on type II diabetic patients, hyperthyroidism and obesity, found the dominance of phyla Bacteroidetes, Firmicutes and Protobacteria in gut microbiota (Wu *et al.*, 2010; Gill *et al.*, 2006; Ley *et al.*, 2005; Zhou *et al.*, 2014). Our results also showed the same phyla Bacteroidetes Firmicutes and Protobacteria in the gut microbiota of asthma patients. According to sequence results, *Klebsiella oxytoca*, found in asthma patients group, which is a gram-negative bacterium that can cause colitis and sepsis (Högenauer *et al.*, 2006).

DGGE has many potential advantages over culture techniques for anaerobic gut microbial analysis. Multiple samples can be analyzed, simultaneously, on a single gel which gives a direct comparison among samples, and dominant bands of interest can be excised and sequenced directly to identify bacterial species or genus. To analyze the molecular characteristics of complex microbial communities, DGGE fingerprint has, now, routinely been used (Ma *et al.*, 2011; Wu *et al.*, 2010; Zhou *et al.*, 2014). Though, DGGE is a semi-quantitative technique, band density results may not accurately relate to the target abundance, hence, there is possibility that a clear associations between species abundance and diseases presence may not necessarily be recognized (Ledder *et al.*, 2007). In our study, some significant and basic gut microbial characterization were established by combining similarity and diversity analysis with dominant DGGE bands excision and with PCR re-amplification of excised bands and sequencing. However, there was a significant disparity in intestinal microbial composition between asthma patients and normal control, established

in the current study was fairly interesting as there was no direct relationship between asthma and intestinal flora. So, present study findings of dissimilar gut bacterial community texture between asthma and control groups shows that alteration of health condition could influence the gut microbial composition.

We also performed real time PCR to observe the accurate quantitative changes of intestinal microbiota (Lyons *et al.*, 2000) and results indicated that, there was significant reduction of *Bifidobacterium* and *Lactobacillus* in diseased group as compared to healthy control which are aligned with previous work of patients with hyperthyroidism in which *Bifidobacterium* and *Lactobacillus* significantly reduced (Zhou *et al.*, 2014). Also, there was a significant decrease of *Clostridium leptum* sub group in asthma patients which is aligned with previous study of *Clostridium leptum* sub group bacteria abundance and diversity in stool microbiota of inflammatory bowel disease patients, furthermore, this shows low level of *Clostridium leptum* sub group causes inflammatory bowel disease (Kabeerdoss *et al.*, 2013). Moreover, there was non-significant increase and decrease of *Peptostreptococcus productus* and *Bacteroides vulgatus* respectively, this non-significant decrease of *Bacteroides vulgatus* in asthma group is also in line with previous studies on diabetes type II patients and metabolic endotoxemia initiates obesity and insulin resistance, which showed non-significant decrease of *Bacteroides vulgatus* (Wu *et al.*, 2010; Cani *et al.*, 2007).

In human, the gut microbiota mainly divides into two groups, Firmicutes and Bacteroidetes (Xu *et al.*, 2007). *Bacteroides vulgatus* is, numerically, predominant species in gut microbiota which indicates a complex but beneficial relationship with the host by prevention of intestinal colonization (Wells *et al.*, 1988; Wexler, 2007). In our routine daily life, the most frequently used probiotics are mainly the *Lactobacillus* and *Bifidobacterium* genera and have health benefits (Butel, 2014). *Bifidobacterium* and *Lactobacillus* are constantly reduced in colorectal cancer (Borges-Canha *et al.*, 2015) and they have also shown anti-atherogenic, anti-inflammatory and anti-obesity effects in number of studies (Nova *et al.*, 2016). Different *lactobacillus* strains have similar moderate antimicrobial activities against pathogens (Shim *et al.*, 2016). While, *Peptostreptococcus productus*, is a reductive acetogenic strain, with its continuous administration, competes with methanogens for H₂ in the gut microbial ecosystem due to its reductive acetogenic character (Nollet *et al.*, 1997).

CONCLUSION

In this study, we observed the change in characterization of gut microbiota of asthma patients. It

also indicates that gut microbiota of asthma patients has some significant changes which is associated with disease occurrence. DGGE analysis shows that a significant change in gut microbial diversity and similarity in disease group as compared to healthy controls. Real time PCR results revealed that in asthma patients, *Bacteroides vulgatus*, *Peptostreptococcus productus* have exhibited the changes of different degree, while copy numbers of *Bifidobacterium*, *Lactobacillus* and *Clostridium leptum* sub group are significantly reduced in asthma group. So, more multicenter studies are required with larger sample size of both patients and healthy subjects to understand the process and mechanism of dysbiosis in gut microbiota with the development of asthma.

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Limitation of study

The smaller sample size is one of the limitations of our study and it is mainly because of the unwillingness of the patients to give their fecal samples. Moreover, excluding the patients and healthy subjects taking other drugs apart from antibiotics, could also improve our understanding about the subject matter.

Statement of conflict of interest

We hereby disclose that we do not have any conflict of interest including any financial, personal or other relationships with other people or organizations.

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