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Gut bacterial microbiota in psoriasis: A case control study

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Gut microbiota is mainly composed of four phyla; however, the human gut microbiota is dominated by only 2 of them and most of them are uncultivable. Psoriasis is an inflammatory skin disorder with associated inflammation of internal organs and musculoskeletal system. This study aimed to, identify numerically abundant bacteria phyla in fecal samples of patients with psoriasis, evaluate whether differences in fecal microbiota correlate with the occurrence of psoriasis and understand the possible pathogenesis behind psoriasis-related bacterial targets. From April, 2015 to 2016, 90 adults were selected prospectively to allocate 2 equal groups: Gr1 (45 cases) patients with psoriasis, and Gr2 (45 cases) healthy controls. Psoriasis Area and Severity Index (PASI) for each psoriasis patient was detected. All subjects were subjected to history taking, clinical examination, and fecal real time polymerase chain reaction (PCR) testing for the Firmicutes, Bacteroidetes, and Actinobacterial phyla. In both groups, Firmicutes were the most common detected phylum followed by Bacteroidetes and finally Actinobacterial phyla. High statistically significant difference was reported for the Firmicutes/ Bacteroidetes ratio between the psoriasis patients and the control group and showed statistically significant positive correlations with PASI. Actinobacterial count was significantly higher in the control group than in psoriasis patients and showed statistically significant negative correlations with PASI. It is believed that, there are fractions of the gut microbiota with the ability to counteract inflammation (Bacteroidetes and Actinobacterial), and others that are more prone to induce inflammation (Firmicutes) and the disturbed microbiome ratio may be the cause for inducing psoriasis.

Key words: Psoriasis, gut microbiota, real time polymerase chain reaction (PCR), firmicutes, bacteroidetes, actinobacteria.

INTRODUCTION

The collection of microorganisms that live in a peaceful coexistence with their hosts has been called the microbiota (Kunz et al., 2009), which colonizes every exposed body surface to the external environment such as skin, genitourinary, gastrointestinal, and respiratory tracts (Chiller et al., 2001; Verstraelen, 2008). The human

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Table 1. Method for calculating the Psoriasis Area and Severity Index (PASI).

Degree of Severity (per body region)	Value given
No symptoms	0
Slight	1
Moderate	2
Marked	3
Very marked	4

Surface involved (per body region)	Value given
<10%	1
10%-29%	2
30%-49%	3
50%-69%	4
70%-89%	5
90%-100%	6

gut has a major surface for microbial colonization and rich in molecules that can be used as nutrients by microbes, making it a preferred site for colonization, so the most heavily colonized organ is the gastrointestinal tract; and the colon alone is estimated to contain over 70% of all the microbes in the human body (Ley et al., 2006). Gut microbiota are mainly composed of four phyla, namely, Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Qin et al., 2010). The human gut microbiota is dominated by only 2 of them: the Bacteroidetes and the Firmicutes (>98%), whereas Actinobacteria, Proteobacteria, and others are present in minor proportions (Eckburg et al., 2005). Gut microbiota provides its host with a physical barrier to pathogens by competitive exclusion, such as occupation of attachment sites, consumption of nutrient, and production of antimicrobial substances. It also stimulates the host to produce various antimicrobial compounds. Healthy gut microbiota is essential to promote host health and disturbance of it results in a variety organ system diseases (Sekirov et al., 2010), as the intestinal microbiome is able to affect extra-intestinal distant sites (Eppinga et al., 2014).

Cultivation has great advantages in microbiology diagnosis; however, traditional bacteriological methods recover less than 40% of the total bacterial species of the GIT, and cultivable bacteria is not a representative of the total phylogenetic diversity (Eckburg et al., 2005). Rapid nucleic acid amplification and detection technologies quickly displace the traditional assays. Real-time PCR techniques with specific 16S ribosomal RNA (rRNA) gene-based oligonucleotide primers had been demonstrated to be powerful methods for detecting target bacteria in complex ecosystems (Layton et al., 2006). Several dermal diseases appear to have a gut-skin connection. Psoriasis is a systemic autoimmune inflammatory disease that shares some immunological aspects with other inflammatory based diseases, such as Crohn's disease (Moran and Shanahan, 2014). GIT disorders are present in 28% of patients with psoriasis (Krinitsina, 1998).

There is growing evidence for a gut-skin connection (Gueniche et al., 2010; Bowe and Logan, 2011). However, this is still an emerging field and there is much research that needs to be conducted, to have a better understanding of the relationship between the gut and the skin. Our hypothesis is that, the immune-mediated inflammatory pathway in psoriasis are induced or mediated by the disturbance in the gut microbiome.

MATERIALS AND METHODS

This study was conducted over the period from February 2015 to February 2016 on 90 adults attending University Hospital after IRB approval and informed consent. They were selected prospectively to allocate 2 equal groups: Gr1 (n = 45) patients with psoriasis (all of the patients had active psoriatic lesions), and Gr2 (n = 45) healthy controls, age, and sex matched individuals with no history of psoriasis. Exclusion criteria were as follows: oral antibiotic, systemic corticosteroids and immunosuppressive therapy within 3 month of sample collection, current extreme diet (e.g., parenteral nutrition or macrobiotic diet), history of IBD, current consumption of probiotics, gastrointestinal tract surgery leaving permanent residua (e.g., gastrectomy, bariatric surgery, or colectomy). Body Mass Index (BMI) was calculated using the following equation: weight (kg)/height (m^2). Subjects with BMI 19 to 25 are considered normal, 26 to 29 as overweight and >30 as obese (Daviglus et al., 2003).

All subjects were subjected to history taking and clinical examination Psoriasis patients were diagnosed in a dermatology clinic, Psoriasis Area and Severity Index (PASI) were recorded for each patient (Fredriksson and Pettersson, 1978), which involves the assessment over 4 body regions (head [h], trunk [t], upper [u] and lower [l] extremities) of erythema (E), infiltration (I), desquamation (D), and body surface area involvement (A), as in Table 1. Since the head, upper extremities, trunk, and lower extremities correspond to approximately 10, 20, 30, and 40% of body surface area respectively, the PASI score is calculated by the formula:

PASI = 0.1 (Eh + Ih + Dh) Ah + 0.2(Eu + Iu + Du)Au + 0.3(Et + It + Dt) At + 0.4(El + Il + Dl)Al

Samples

Fecal samples were collected from all participants in sterile collection containers. Undigested particles approximately 1 g stool were removed by washing, low-speed centrifugation of samples, followed by high centrifugation of the supernatant were used to form bacterial pellet which were stored frozen at –70°C until use.

Extraction of gut microbiota DNA

DNA was obtained from the samples bacterial pellet by QIAamp DNA Stool kit, according to the manufacturer's instructions (QIAGEN, Hilden, Germany).

Table 2. Specific primers pairs sequences used.

Target phylum	Target gene	Primers sequences	References
Total bacteria	16S rRNA	FP: ACTCCTACGGGAGGCAGCAG RP : ATTACCGCGG CTGCTGG	Fierer et al., 2005
Firmicutes	16S rRNA	FP: GGAGYATGTGGTTTAATTCGAAGCA RP:AGCTGACGACAACCATGCAC	Guo et al., 2008
Bacteroidetes	16S rRNA	FP: GGARCATGTGGTTTAATTCGATGAT RP: AGCTGACGACAACCATGCAG	Guo et al., 2008
Actinobacteria	16S rRNA	FP:TACGGCCGCAAGGCTA RP: TCRTCCCCACCTTCCTCCG	Bacchetti et al., 2011

Real time PCR

The real time PCR was carried out to detect the copy numbers of the 16S rRNA gene for all Bacteria, three dominant phyla were present in the gut by using specific primers which include: most Bacteroidetes, Firmicutes, and Actinobacterial division, as shown in Table 2. Amplification and detection of DNA by real-time PCR were performed with ABI-Prism 7500 Sequence Detection System (Applied Biosystems) using optical grade 96-well plates. Duplicate sample analysis was performed, in a total volume of 25 µl using SYBR Green PCR Master Mix (QIAGEN, Hilden, Germany). Each reaction contained 12.5 µl of SYBR green master mix, 0.3µl of each primer, and 2.5 µl of the DNA template. The PCR reaction conditions consist of an initial denaturation step of DNA at 95°C for 10 min followed by 40 cycles consisting of denaturation at 95°C for 15 s, and annealing-elongation step at 60°C for 1 min (Guo et al., 2008). A melting curve analysis was done, and the threshold cycle (CT) values and baseline settings were determined by automatic analysis settings. The data were analyzed using the Sequence Detection Software version 1.6.3 (Applied Biosystems).

Standard curves

Standard curves were generated by using 10-fold serial dilutions, (5X1015 to 5x106) of chimeric plasmid which was quantified using a spectrophotometry (NanoDrop ND-1000, USA) (Armougom et al., 2009). Each standard curve was generated by plotting Ct values versus the number of plasmid copies. When PCR was performed on tested fecal samples, we used this standard curve to quantify each bacterial phylum. The chimeric fragment sequence (362 bp) was constructed, and it contains parts of 16S rRNA genes of Bacteroidetes (sequence is in regular style), and Firmicutes (sequence is in bold style). The primers sequences were complementary to that in lowercase underlined: agcagccgcggtaatACGGAGGATCCGAGCGTTATCCGGATTTATT*GGGTTTAAGGG*AGCGTAGGTGGACTGGTAAGTCAGTTGTGAAA GTTTGCGGCTCAACCGTAAAATTGCAGTTGATACTGTCAGTCT TGAGTACAGTAGAGGTGGGAATTCGTGGTgtagcggtgaaatgcttagg tcagctcgtgtcgtgaGATGTTGGGTTAAGTCCGCAACGAGCGCAACC CTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGAC TGCCGGTGACAAACCGGAGGAAGGTGGGGATGAC*GTCAAATC ATCATGCC*CCTTATGACCTGGGCTacacacgtgctacaatgg

Statistical analyses

The data were statistically analysed using the Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data were presented as, means and standard deviation and also described as numbers and percentages. Mann-Whitney test was used for comparing groups. All differences were considered statistically significant if $p<0.05$. Correlations were established by coefficient of correlation (r).

RESULTS

Patients with psoriasis and healthy control subjects, were matched in age (42.3 ± 10 years, vs 44.2 ± 7.1, respectively), and sex (27 females /45patient, vs 25females/45 subjects, respectively). No statistically significant difference was observed between the two groups regarding BMI (24.5 ±4.7, vs26.9 ±7.1, respectively). Firmicutes was the commonest detected phyla in psoriasis patients (83%), while Bacteroidetes and Actinobacterial phyla were accounted for 9.2 and 2.8%, respectively. In the control group the detected percentage were 70, 22, and 3.9% for Firmicutes, Bacteroidetes, and Actinobacterial phyla, respectively. There was non-significant difference between patients in GpI and GpII for total bacteria, Firmicutes and Bacteroidetes phyla counts. However, Actinobacterial phylum count was significantly ($p < 0.001$**) higher in the control group than in psoriasis patients, as in Table 3 and Figure 1.

For Firmicutes/Bacteroidetes ratio (F/B), we observed a high statistically significant difference ($p < 0.001$**) between the ratio in psoriasis patients (9.02) and in the control group (3.18), as showed in Figure 1. The mean value for PASI was 11± 9, and showed a statistically significant positive correlations with Firmicutes/ Bacteroidetes ratio (r=0.312, p=0.036*).

Statistically significant negative correlations were observed with Actinobacterial phylum (r=-0.298, p=0.047*). Non-significant correlations were detected with Firmicutes (r=0.264, p= 0.079), and Bacteroidetes (R=-0.292, P= 0.052). However, weak negative correlation was reported with Bacteroidetes.

Table 3. Comparison betweengut microbiota gene copies number/gm stool in GpIand GpII.

Phyla	GPI	GpII	P
All bacteria	1.1 × 1012±19.5	1.3 × 1012±16.88	0.96
Bacteroidetes	1.012 × 1011±8.2	2.86 × 1011±8.7	0.3
Firmicutes	9.13× 1011±7.62	9.1 × 1011±10.07	0.99
Actinobacterial	3.12 × 1010±1.46	5.06 × 1010±1.54	<0.001**

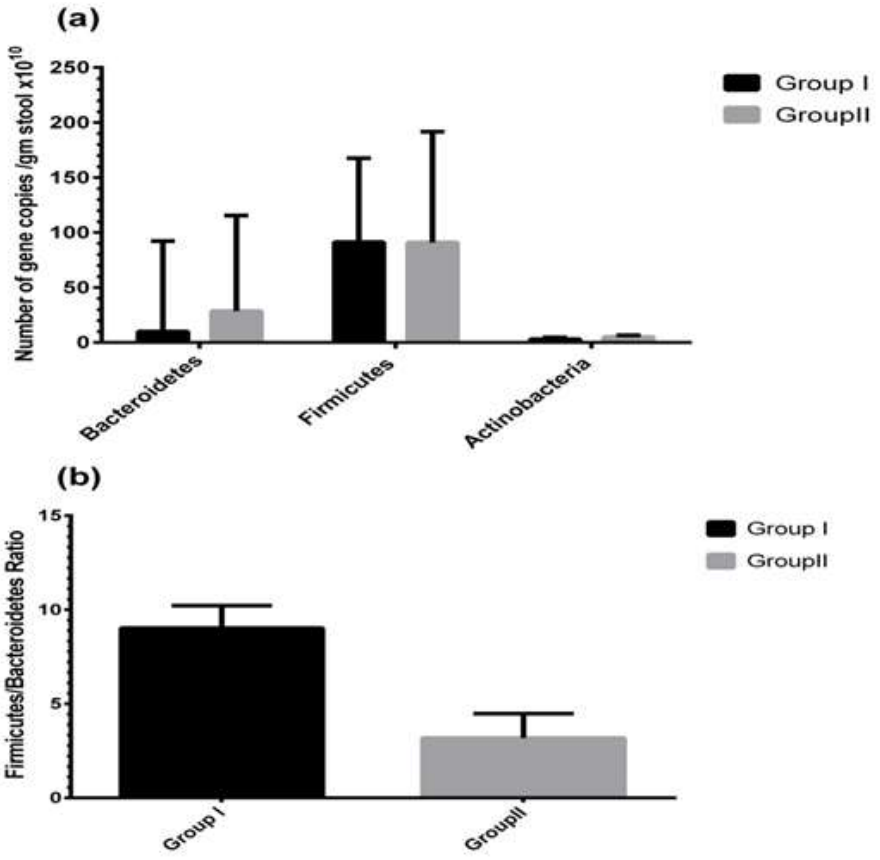

Figure 1. Box-and-Whisker plot of A) Gut microbiota gene copies number/gm stool in GpI and GpII b) The Firmicutes/Bacteroidetes ratio in GpI and GpII.

DISCUSSION

In the current study, in both GpI and GpII, Firmicutes was the commonest detected phyla (83 and 70%, respectively), followed by Bacteroidetes (9.2 and 22%, respectively). It was clear that both Firmicutes and Bacteroidetes were the dominant phyla in gut microbiome and accounted for more than 90% of the total detected bacteria (92.2% in GpI and 92% in GpII). These results were parallel to Rajilic-Stojanovic et al. (2007) who reported that Firmicutes and Bacteroidetes phyla were predominating and represent 90% of the total gut microbiota (that is, 65 and 25%, respectively). A lower percentage (80%) was reported for the three phyla by Lay et al. (2005). In contrary, Manson et al. (2008) reported a higher percentage (99%) for the two dominant intestinal microbiome phyla, also Marchesi (2010) mentioned that the large majority of microbes reside in our GIT belong either to the Firmicutes or Bacteroidetes phyla and that was similar to Koenig et al. (2011) results, who reported about 80 and 20% for Firmicutes and Bacteroidetes, respectively. This variation is accepted as

these proportions can vary greatly between individuals and even within single individual over time because peoples received antibiotic and reduced certain bacterial types according to the used antibiotic. Besides that, the dietary changes can influence the types of microbiota, as polysaccharide-rich diet significantly altered the microbiota composition, resulting in an increase in Firmicutes and decrease in Bacteroidetes, while high fiber diet has been associated with increases in Bacteroidetes and a lower abundance of Firmicutes in humans (Turnbaugh et al., 2009; Hakansson and Molin, 2011). In the present study, there was no significant difference between patients in GpI and GpII for total bacterial, Firmicutes and Bacteroidetes phyla counts. However, it was reported that, a highly significant difference was observed between the F/B ratios and was higher in psoriasis patients than in the control group. Also PASI showed statistically significant positive correlations with F/B ratio in spite of non-significant correlations that were observed with Bacteroidetes, and Firmicutes alone. It should be borne in mind that certain combination of the microbiota can enhance the pathogenic effects, and another combination can keep the person healthy, so it's all about the ratio of certain microbiota that have a beneficial effect when compared with the ratio of others that disturb the balance.

In some chronic diseases, as psoriasis, the pathologic agent might be the disturbed microbiota ratio, and this presumably means a decreased bacterial diversity and/or different degrees of overgrowth by bacteria inducing inflammatory responses by the immune system (Hakansson and Molin, 2011; Fry et al., 2013). The question is which bacteria are the most forceful in causing inflammation? Let's remember that CD4+ T helper cells can differentiate towards Th1, Th2, Th17 and Treg phenotypes (Gaboriau-Routhiau et al., 2009). Zambrano-Zaragoza et al. (2014) detected that the dys regulation of IL-17 (pro-inflammatory) has been implicated in psoriasis, while Tregs have an anti-inflammatory role, and its increase is an early predictive marker for clinical response in psoriasis as reported by Richetta et al. (2011). Some gut bacteria appear to drive Treg development preferentially, while others promote Th17 development (Ishikawa et al., 2008). Gut microbiota has a significant influence as a complex, on the development of inflammatory/autoimmune diseases but the specific mechanisms that lead to the induction of Tregs versus Th17 cells by various commensal bacteria are currently unknown. However, toll-like receptor 9 plays a major role (Dalpke et al., 2006). Mazmanian reported that B. fragilis (Bacteroidetes phylum) increases the suppressive capacity of Tregs and induces it to produce anti-inflammatory cytokine (Mazmanian et al., 2008).

Moreover, Oral administration of antibiotic which leads to a reduction in bacteria from the Firmicutes phylum, and a relative increase in bacteria from the Bacteroidetes phylum, suggested that antibiotic-mediated protection against inflammation mediate the increase in Bacteroidetes with enhancement of dendritic cells that converted the naïveTcells into IL-10-producing Tregs (Ochoa-Reparaz et al., 2010). Most of the Firmicutes are Gram positive bacteria, while most of the Bacteroidetes are Gram negative bacteria (Hakansson and Molin, 2011). Firmicutes was the most common phylum of the skin in psoriasis. Fry suggested that Crohn's disease (CD) occurs as a result of immune tolerance breakdown of the intestinal microbiota in genetically susceptible individuals and he also reported that CD patients are 5 times more likely to develop psoriasis, so psoriasis may occurs due to immune tolerance breakdown of microbiota (Fry et al., 2013). Many studies reported that the peptidoglycan (PG) is antigenic and triggers psoriasis, in which the T cell stimulation which occurs is proved by isolated PG-specific T cells from psoriatic skin lesions, furthermore there are four Peptidoglycan recognition proteins (PGRPs), which bind to the PG of Gram-positive bacteria. PGRP-3 and PGRP-4 are secreted in the gut, and skin. In psoriasis there are mutations in the genes for the PGRP-3 and PGRP-4 and this may lead to an abnormal response to bacterial PG in psoriasis, which may results in inflammation (Kainu et al., 2009; Dziarski and Gupta, 2010). In the current study, Actinobacterial phylum accounted for 2.8% in GpI, and 3.9% in GpII and showed a high statistically significant difference between the two groups. This was close to the results of Koenig et al. (2011), who reported that Actinobacterial phylum was 3% of the total microbiome in the gut. Also PASI showed statistically significant negative correlations with Actinobacterial phylum. This is in harmony with many studies who illustrated the complex relation between Actinobacterial phylum and inflammatory or immune related diseases as what we have here in psoriasis (Veiga et al., 2010; Kosiewicz et al., 2011). Studies reported that oral administration of probiotics, including the Bifidobacterium species (Actinobacterial phylum) reduced the intestinal inflammation in the colitis model and protect it against the development of various inflammatory and autoimmune diseases by, reducing the levels of other bacteria that cause inflammation. The authors also reported that the fecal levels of Bifidobacterium were inversely related to the inflammatory core (Calcinaro et al., 2005; Lavasani et al., 2010).

The inflammation-suppressing fractions of the microbiota may: (i) counteract some of the inflammation-aggravating bacteria, which will decrease the inflammatory tone of the system; (ii) improve the barrier effect of the GI mucosa, which allows less inflammation-inducing components in the lumen to translocate out into the body; (iii) directly interact with inflammation-driving components of the immune system. All three actions may be at work simultaneously (Fry et al., 2013). The limitations of this study are: relatively small sample size,

un-attainability of detecting the skin microbiome to explore the relation between the detected types of gut microbiome and skin microbiome and to clear the effect of gut microbiome in psoriasis skin plaques.

Conclusion

Firmicutes and Bacteroidetes were the dominant phyla in gut microbiome. F/B ratio was higher in psoriasis patients group than in the control group which showed positive correlation with PASI. It is believe that, there are fractions of gut microbiota which has the ability to counteract inflammation, and others which are more prone to induce inflammation (Firmicutes). The disturbance of microbiota ratio may be the cause for inducing inflammatory responses by the immune system in psoriasis. Actinobacterial group may be one of the bacteria that play an anti-inflammatory role as it was decreased significantly in psoriasis patients and negative correlations were reported with PASI. However, the relationship between psoriasis and gut microbiome should be dealt with caution for further investigations.

Conflict of Interests

The authors have not declared any conflict of interests.

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