Research Article

Isolation and Characterization of Aerobic Gut Microbiome of Psoriatic Arthritic and Psoriasis Patients

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ABSTRACT

Background and Objectives: Psoriatic arthritis (PsA) and Psoriasis (Ps) is a chronic inflammatory disorder affecting mainly the skin and synovial membrane. While the etiology remains underexplored, multiple factors seem to play a role in pathogenesis. Reports show that the gut microbiome contributes to arthritis and related inflammation of the joints.

Material and Methods: This study was initiated to understand the variation in the microbiome in PsA patients visiting the Mahatma Gandhi Medical College and Research Institute (MGMCRI) hospital, Puducherry. Fecal samples were collected from 15 clinically and diagnostically confirmed as PsA and Ps patients. They were subjected to standard microbiological analysis.

Results: Gram positive *Staphylococcus aureus* and *Streptococci* constituted 30% of the isolates. Remaining 70% included Gram negative bacteria with a predominant presence of *Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Proteus mirabilis.*

Conclusion: A total of 30 aerobic bacterial isolates were identified from the fecal samples of PsA and Ps Patients visiting MGMCRI hospital. To the best of our knowledge, this is the first South Indian study characterizing the fecal microbial compositions in PsA patients.

Key words:
Psoriatic arthritis, Psoriasis, Inflammation, Fecal sample, Bacteria, Gut microbiome.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease, associated with chronic inflammatory skin disease Psoriasis (Ps) and belonging to the family of Spondyloarthritis[1,2] Mostly Ps develops first followed by PsA. One-third of patients with Ps are diagnosed with PsA after about 30 years leading to history of Ps.[3] The etiology is still not completely studied. Genetic, immunological, and environmental factors contribute to the development of PsA[4].Previous studies point out that PsA is triggered by viruses, bacterial infection, trauma or stress. Interestingly the role of gut microbiome is emerging as a strong etiology, and associated gut inflammation, in the pathogenesis of the Ps and PsA disease spectrum[5]. HLA-B27 over-expressed rats, for instance, develop arthritis and colitis only in the presence of specific intestinal microbes.[6] Epidemiologic evidence also suggests a relationship between gut microbiome and joint inflammation in PsA.[7-10]

Among sites of exposure to bacterial antigens, the intestinal mucosa represents a unique environment for triggering of local and distal autoimmunity.[11]Earlier studies have documented that ß-hemolytic *streptococcal* infection may precipitate guttate psoriasis in young children.[12] Decreased level of *Propionibacterium* in psoriasis patients may be caused by increased composition of *Streptococcus* species compared to controls. Composition of gut microbiome, the ratio of *Firmicutes* and *Bacteroidetes* was unsettled in psoriatic individuals compared to

healthy controls. *Streptococcal* infection alters the cutaneous floral composition that may lead to the onset of Ps.[13] Reports are available correlating the role of infectious agents including *Shigella*, *Salmonella*, and other gram-negative microorganisms which cause the decreased diversity of transient microflora present in the host that may lead to the onset of inflammatory diseases including PsA.

In this study we aim to analyze aerobic bacterial composition from the fecal samples of PsA and Ps patients visiting MGMCRI hospital.

MATERIAL AND METHODS

Study participants

Subjects visiting Department of Dermatology, Venereology and leprology, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Puducherry were clinically evaluated for the presence of PsA, from November 2015 to June 2106. Each patient's medical history and medications were noted and reviewed. All the patient details were obtained, reviewed and grouped, from laboratory and radiographic reports, from the hospital records. A total 15 subjects were randomly chosen according to the criteria of Wright and Moll.[14] All PsA patients were in one of the following three main categories; axial disease, oligoarthritis, polyarthritis. This study was approved by the Institutional Human Ethical Committee (IHEC) of MGMCRI Puducherry, and written informed consent was obtained from all study participants as approved.

Inclusion and exclusion criteria

The subjects of the study, above 18 years old, were chosen based upon the classified score of values of Psoriasis Epidemiology Screening Tool (PEST) and Psoriasis Area and Severity Index (PASI). PASI score of each patient was determined using the free online software "Psoriasis Area Severity Index (PASI) Calculator" (version 1.7.1, URL: http://pasi.corti.li/#ref4). PASI score combines the severity and percentage of affected area. It was calculated on a scale from 0 to 4 by expressing the severity of psoriasis, including erythema, induration and desquamation. Psoriasis Epidemiology Screening Tool (PEST) is a validated questionnaire-based screening tool for PsA. It is recommended for subjects with psoriasis who do not have a diagnosis of PsA (NICE psoriasis guidelines 2012).[17] Each individual having 3 or more PEST score are indicative of PsA.

The patients were having current active psoriasis of the skin (Ps) and arthritis. Exclusion criteria were recent (<3 months prior) use of any systemic disease-modifying anti-rheumatic drugs (DMARDs, oral and/or biologic agents) or steroids, antibiotic therapy, current extreme diet (e.g., parenteral nutrition or macrobiotic diet), known history of malignancy or irritable bowel diseases, current consumption of probiotics, or any gastrointestinal tract surgery leaving permanent residua (e.g., gastrectomy, bariatric surgery, colectomy).

Sample collection

Patients were educated in fresh fecal sample collection procedure. Within 30 min of defecation, samples were collected in sterile stool container, and processed within one hour of sample collection.

Isolation of enteric bacteria

One gram of sample was mixed with 99 ml of sterile peptone water in 250 ml conical flask, homogenized until a uniform suspension was obtained and serially diluted (10^{-1}-10^{-6}). 0.1 ml quantity of highest dilution was plated in the blood agar, nutrient agar, and MacConkey agar media and incubated aerobically for 24-48 hours.

Colonies were picked based on their morphology from respective plate and purified twice by streaking on the nutrient agar and preserved in the nutrient agar slant for short term uses. Stock cultures were preserved in Nutrient broth supplemented with 15% (v/v) glycerol at -80°C for further examination. Cultures were activated prior to testing by subculturing twice in the Nutrient broth.

Characterization and Identification

Isolates were microscopically examined for gram stain and motility. Catalase test was done by using freshly prepared 3% H_2O_2. H_2S and gas production from glucose was performed by using TSI agar slants, incubated both at 10°C and 45°C. Isolates were subjected to a battery of biochemical tests: Catalase, oxidase, Indole, Methyl red, Voges-Proskauer, Citrate (IMViC) and different sugar fermentation tests. All the media were purchased from Himedia Laboratories. All the above tests were conducted in triplicates for each isolate. Bacterial strains were identified following the taxonomic keys of Bergey's Manual of Systematic Bacteriology. [15, 16]

Table 1: Demographic and clinical data of study participants with recent-onset psoriatic arthritis (PsA)

S.No	Age (Years)	Sex	Duration of the disease (months)	Lesion sites	PEST Score	PASI Score	Conformation
1	27	M	09	T/ULL	4	5.6	PsA
2	61	M	120	T/H/ULL	4	13.1	PsA
3	56	F	30	ULL	3	2.8	PsA
4	38	M	03	T/ULL	2	6.7	Ps
5	50	M	108	H/T	3	7.2	PsA
6	48	F	72	H/T/UL	4	6.9	PsA
7	52	M	120	T/ULL	3	12.1	PsA
8	36	F	04	H/T/LL	3	7.8	PsA
9	63	M	06	H/T/UL	3	8.9	PsA
10	66	M	54	ULL	3	15.6	PsA
11	47	M	132	H/T/ULL	3	14.8	PsA
12	35	M	03	H/ULL	1	5	Ps
13	35	M	06	H/T/LL	2	5.8	Ps
14	70	M	36	H/T	3	7.4	PsA
15	49	F	156	H/T/UL	4	20.01	PsA

Abbreviations: PsA- psoriatic arthritis, PEST- Psoriasis Epidemiology Screening Tool, PASI- Psoriasis Area and Severity Index, T-Trunk, H-Head, ULL-Upper and lower limbs, LL-Lower limbs, UL- Upper limbs

RESULTS

The details of age, sex, duration of the disease periods and lesion sites (head, arms, trunk and legs) of the study participants were noted. The patient demographic details and PEST and PASI scores are given in Table 1

Phenotypic methods analysing the morphological and biochemical characteristics and identification of aerobic bacterial gut flora from the patients are given in Table 2.

A total of 30 culture-confirmed PsA and Ps are reported here. Only those isolates which are predominant and in significant numbers from stool culture plates were taken up for further processing. The percentage of bacterial population showed variation. The majority of bacterial species included *E. coli* (27%), *Staphylococcus aureus* (23%) and *Klebsiella pneumoniae* (20%), whereas other bacteria like *Enterococcus faecalis, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus mirabilis* and *Micrococcus ovalis* were of lesser percentage namely 10, 7, 7, 3, 3 as listed in Table 2.

DISCUSSION

In this study we have documented the presence of 30 aerobic gut microbiome in clinically defined PsA and Ps symptoms. Previous studies conducted elsewhere have concluded a similar relationship between arthritis and infection with *Salmonella*, [18,19] *Shigella*, [20,21]. *Salmonella* infection associated with rheumatological illness is also reported.[22] *E. coli* pathogen linked to reactive arthritis with genetic similarities of *Shigella* species[23]. Skin appearances are often associated with systemic autoimmune diseases including PsA caused by *E. coli*[24]. *Staphylococcus aureus* colonization in patients with psoriasis have high relative risk to develop inflammatory reaction.[25] Antimicrobial peptide (AMP) is normally produced by keratinocytes in human skin in response to inflammatory stimuli such as psoriasis. Colonization of *Staphylococcus aureus* reduces the high expression of AMP in psoriasis skin thereby reducing the risk of skin infections.[26] In this study, we have identified 23% incidence of *S. aureus*. This is significantly higher compared to

Table 2: Bacterial pathogens isolated from stool samples of PsA and Ps Patients

Name of Pathogens	No. of Strains	Percentage
E. coli	08	27
Klebsiella pneumoniae	06	20
Staphylococcus aureus	07	23
Streptococcus pyogenes	02	07
Pseudomonas aeruginosa	02	07
Proteus mirabilis	01	03
Micrococcus ovalis	01	03
Enterococcus faecalis	03	10

all other samples. *Klebsiella pneumoniae* plays a major role in inducing inflammation. *K. pneumoniae* utilize iron from its host by secreting siderophores, small iron-chelating molecules and these siderophores are believed to worsen infections by promoting bacterial growth[27]. *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis, Streptococcus pyogenes, Pseudomonas aeruginosa, Micrococcus ovalis and Proteus mirabilis* were positive in 27%, 20%,10%, 7%, 7%, 3%, 3% respectively in PsA patients studied.

To conclude, a total of 30 isolates were identified by morphological and biochemical methods, from fecal samples of PsA and Ps patients. These fecal samples contained 30% of gram positive bacteria. Amongst them, the predominant species are *Staphylococcus aureus* and *Enterococcus faecalis*. Remaining 70% included gram negative bacteria with a predominant presence of *Escherichia coli* and *Klebsiella pneumoniae,* followed by *Pseudomonas,* and *Proteus mirabilis.* To the best of our knowledge, this is the first South Indian study characterizing the fecal microbial compositions in PsA and Ps patients.

CONFLICTS OF INTEREST

None.

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Benefits of non-surgical periodontal treatment in patients with type 2 diabetes mellitus and chronic periodontitis: A randomized controlled trial.

(Improving dental hygiene could play a significant part in better managing type 2 diabetes)?

A study evaluated the effect of non-surgical periodontal treatment on serum HbA1c levels in patients with type 2 diabetes. This was a 6-month, single-masked, randomized clinical trial based on 90 patients (HbA1c: 7.7% (61 mmol/mol) ± 1.13%) who were randomly assigned to either the treatment group (oral hygiene instructions + scaling and root planing using ultrasound and Gracey curettes) or the control group (oral hygiene instructions + supragingival removal of plaque and calculus using ultrasound). Pocket depth, gingival index, and plaque index were assessed at baseline and after 3 and 6 months together with determinations of fasting plasma glucose, HbA1c, and bacterial counts. Treatment significantly improved the periodontal and metabolic parameters (p <0 .05), whereas in the control group no improvement was observed. These results were consistent with the bacteriological results in most but not all cases.

Conclusion: Non-surgical periodontal treatment resulted in a better glycaemic status of type 2 diabetes patients and demonstrated the importance of oral health in their general health.

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