Alterations of the Human Gut Microbiome in Patients With Hidradenitis Suppurativa: A Case-control Study and Review of the Literature

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Key words: hidradenitis suppurativa, inflammatory skin disorders, gut microbiome, pathogenesis

Citation: Demirel Öğüt N, Hasçelik G, Atakan N. Alterations of the human gut microbiome in patients with hidradenitis suppurativa: a case-control study and review of the literature. Dermatol Pract Concept. 2022;12(4):e2022191. DOI: https://doi.org/10.5826/dpc.1204a191

Accepted: March 14, 2022; Published: October 2022

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Funding: This study was supported by the Turkish Society of Dermatology (Grant 2018/179) and Hacettepe Dermatology Association (Grant 2019/1).

Competing interests: None.

Authorship: All authors have contributed significantly to this publication.

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ABSTRACT

Introduction: Hidradenitis suppurativa (HS) is a chronic and systemic inflammatory disease that extends beyond the skin. The role of gut microbiome (GM) alterations in the pathogenesis of inflammatory and autoimmune disorders is remarkable.

Objectives: Based on the hypothesis that dysbiosis in the GM may trigger systemic inflammation in the pathogenesis of HS, this study aimed to investigate whether the GM is altered in HS patients compared with healthy subjects.

Methods: In the present case-control study, fecal samples from 15 patients with HS and 15 age- and sex-matched healthy individuals were collected and analyzed using 16S rRNA-based metagenomic analysis, New Generation Sequencing (NGS). The V3 and V4-hypervariable regions of the bacterial 16S rDNA gene were amplified from all samples and sequenced by the Illumina MiSeq platform. Bioinformatics analyses were performed in QIIME2.

Results: Shannon alpha diversity index showed significantly reduced diversity in HS patients (P = 0.048). Bray-Curtis Dissimilarity and Jaccard Distance revealed that the gut microbial composition of HS patients was significantly distinctive from that of controls (P = 0.01 and P = 0.007, respectively). The relative abundance of unclassified Clostridiales, unclassified Firmicutes, and Fusicatenibacter in HS was significantly lower than that in controls (P = 0.005, P = 0.029, and P = 0.046, respectively).

Conclusions: This study indicated that significant alterations in the GM of HS patients could play a critical role in the pathogenesis of HS and might be a trigger for systemic inflammation. Increased understanding of the pathogenesis of HS will shed light on the new potential therapeutic targets and novel treatment options.
Introduction

Hidradenitis suppurativa (HS) significantly impacts patients quality of life with its chronic and relapsing course and sub-optimal response to therapy [1]. The etiopathogenesis of the disease has not been elucidated yet. However, it is clear that HS is not simply an inflammatory skin disorder, but a systemic inflammatory disorder [2]. Its associations with inflammatory and autoinflammatory disorders, increased levels of inflammatory cytokines in sera of patients, and γ-secretase complex mutations leading to impaired notch signaling pathway may suggest a systemic inflammation that distant skin sites might trigger [3].

Recently, a term called “gut-skin axis” has been introduced and highlighted a potential link between the gut microbiome and the skin through complex immune mechanisms [4]. The gut microbiome refers to the whole genome of the residential microbial community in the human intestine. An altered gut microbiome is involved in the development of multiple immune-mediated and inflammatory disorders, especially of inflammatory bowel diseases (IBD), by disrupting the balance between pro-inflammatory and anti-inflammatory/regulatory immune cells [5,6]. Many studies have revealed dysbiosis, alterations in the gut microbiome, in inflammatory and autoimmune skin disorders such as acne, rosacea, atopic dermatitis, and psoriasis [7-10]. However, studies exploring the gut microbiome alterations in patients with HS are scarce in the literature [11-14].

Objectives

In the present study, we hypothesize that the gut microbiome of patients with HS is different from that of healthy people. Therefore, in this study, we aimed (1) to evaluate the gut microbiome of patients with HS, (2) to compare it with healthy subjects, and (3) to evaluate the relationship between fecal microbiome and obesity, smoking status, and treatment condition of HS patients.

Materials and Methods

Study Population

The study was conducted over the period from August 2018 to May 2019 at the Dermatology Department in Hacettepe University and enrolled 30 participants, including 15 patients with HS and 15 age- and sex-matched healthy controls without HS and any other skin diseases. Patients with HS and healthy subjects who were between the ages of 18-65 years, without any systemic antibiotic therapy, probiotics, or prebiotics use in the last 3-months, who were not on a current specific diet (vegan, vegetarian, gluten-free diet), who did not have concomitant systemic inflammatory disease, any infections, previous gastrointestinal tract surgery, and malignancy were included in the study. The diagnosis of HS was based on clinical criteria [15]. The severity of the disease was evaluated by Hurley Stage, modified Sartorius Score, and International Hidradenitis Suppurativa Severity Score System (IHS4) [16]. Patients and healthy controls were not given dietary restrictions before stool sampling. In order to assess the possible effect of obesity, smoking status, and current treatment on gut microbiome profile, fecal samples of HS patients were stratified by body mass index (BMI), smoking, and current treatment status.

This study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Board [24.07.2018, GO 18/62925]. Written informed consent was obtained from all subjects prior to their enrollment.

Fecal Sample Collection

Fecal samples from all participants were collected in sterile containers. Then, all samples were transferred to a freezer within 60 minutes of collection and stored at −80°C until analyzed with New Generation Sequencing (NGS).

DNA Extraction

Total DNA extraction from 30 fecal samples was performed using The Biospeedy® DNA Isolation Kit (Bioeksen R&D Technologies) according to the manufacturer instructions. Feces (200 mg) was transferred to 300 µL of buffer (200 mM Tris-HCl, pH 8.0; 20 mM EDTA; 10% Triton X-100) and 0.1-mm glass bead containing tubes and homogenized at 6000 rpm for 1 min. 0.1 µL lysozyme (200 µg/µL) was added to the sample and incubated at 37°C for 15 minutes. Subsequently, 250 µl lysis buffer (0.5 µg /µl Proteinase K, 5% Tween® 20, 3M Guanidinium thiocyanate, 20 mM Tris-HCl, pH 8.0) was added to the sample and incubated at 70°C for 15 minutes and then 95°C for 5 minutes. After incubation, one volume of isopropanol was mixed with the sample, passed through silica columns by centrifugation, and washed twice with washing buffer (20 mM NaCl, 2 mM Tris-HCl, pH 7.7, 80% v/v ethanol). DNA was eluted with 50 µL 100 mM Tris-HCl (pH) and stored at -20°C until analyzed. Spectrophotometric methods measured the amount and quality of isolated DNA in samples, and their suitability to the next steps was tested. Next molecular processes were performed with DNA with OD260 / OD280 ratio of 1.8-2.0 and OD260 / OD230 ratio of 2.0-2 and at least 10 ng/µL (preferably 50-300 ng / µL) concentrations.

16S rRNA Amplification

The amplification of the V3 and V4-conserved regions of bacterial 16S rRNA were performed by polymerase chain reaction (PCR) using Illumina adapter overhang nucleotide 16S rRNA-specific sequences,
Bioinformatics and Statistical Analyses

The raw sequence data were processed using QIIME2 v2019.1 software. First, the barcode and the primers were trimmed, and unique sequences were identified. Pre-clustering prevented redundancy. UCHIME was used for the removal of chimeras. Next, the sequences were classified using a classifier within QIIME2. The references and taxonomy files were obtained from the QIIME2 database. After picking operational taxonomic units (OTU) at 97% sequence similarity and taxonomic assignments using the QIIME2 and SILVA rDNA database, the OTUs were binned into phylotypes.

Alpha diversity and beta diversity were calculated with QIIME2 v2019.1 software. The alpha diversity is defined as the diversity within a community and was calculated using Shannon, Simpson’s, ACE, Chao, and Faith Phylogenetic diversity indices. The beta diversity is defined as the distance between communities and was calculated by Bray-Curtis dissimilarity and Jaccard distance and represented in a three-dimensional Principal Coordinate Axis on EMPeRor. Statistical analyses were performed using Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, United States) and MINITAB 17 software (Minitab Ltd. Co., Coventry, UK).

Results

The Characteristics of Participants

We enrolled 15 patients with HS and 15 age- and sex-matched healthy controls in this study. All subjects were Turkish in origin and coming from exact geographical locations. The characteristics of study groups are shown in Table 1.

Fecal Microbiome Analysis Between HS and Healthy Groups

Community Richness and Diversity

The alpha-diversity of the gut microbiome was significantly lower in HS patients than healthy individuals (Shannon index, P = 0.048). The Simpson, ACE, Chao, and Faith phylogenetic diversity indices of HS patients were lower than those of controls; however, the differences were not statistically significant (P > 0.05) (Figure 1).

The gut microbiome composition in HS patients was significantly distinct from healthy controls. The results of Bray-Curtis and Jaccard Dissimilarity indices showed significantly different clustering of HS patients and healthy subjects based on PERMANOVA statistical analyses (P = 0.01 and P = 0.007, respectively), indicating that the bacterial community structure in HS is different from that in healthy controls (Figure 2).

Overall Taxonomic Analysis of HS Patients and Controls, Distribution at the Phylum Level

The gut microbiome of both HS patients and healthy controls was largely dominated by Firmicutes (relative abundance 92.64% vs. 93.10%), Bacteroidetes (3.53% vs. 4.64%), Actinobacteria (0.95% vs. 1.23%), unclassified Bacteria (0.4% vs. 0.54%), Proteobacteria (0.52% vs. 0.4%), and Verrucomicrobia (0.52% vs. 0.4%). The proportions of rare phyla including Synergistetes, Tenericutes, Fusobacteria, Candidatus_Saccharibacteria, Acidobacteria, Chloroplast, and Cyanobacteria were present at much lower levels. Firmicutes was the most predominant phylum among all relatively abundant dominant taxa in HS and healthy controls. The overall microbial composition of each group at the phylum level is represented in Figure 3. The phylum unclassified Bacteria was significantly reduced (P = 0.032) in the HS group compared to the healthy control, whereas other phyla showed no significant difference.

Overall Taxonomic Analysis of HS Patients and Controls, Distribution at the Genus Level

Lachnospiraceae_unclassified, Ruminococcaceae_unclassified, Clostridiales_unclassified, Roseburia, Gemmiger,
Table 1. Characteristics of hidradenitis suppurativa patients (N = 15) and healthy individuals (N = 15) included in the study.

| Characteristic                          | HS patients (N = 15) | Healthy controls (N = 15) | P     |
|----------------------------------------|----------------------|--------------------------|-------|
| Male / Female (N)                      | 10 / 5               | 10 / 5                   |       |
| Age, years, mean ± SD (range)          | 33.23 ± 12.29 (18-57)| 33.23 ± 12.29 (18-57)   |       |
| BMI, mean ± SD                         | 28.63 ± 5.7          | 24.7 ± 2.37              | 0.026a|
| Healthy weight (N)                     | 5                    | 11                       |       |
| Overweight (N)                         | 3                    | 3                        |       |
| Obese (N)                              | 7                    | 1                        |       |
| Cigarette pack year, mean ± SD         | 13.13 ± 11.79        | 4.93 ± 9.97              | 0.011a|
| Current smoker (N)                     | 13                   | 4                        |       |
| Non-smoker (N)                         | 2                    | 11                       |       |
| Disease onset, years, mean ± SD (range)| 24 ± 9.9 (15-48)     | -                        |       |
| Family history (N)                     | 5 / 15               | -                        |       |
| Hurley stage (N)                       | -                    | -                        |       |
| I                                      | 2                    | -                        |       |
| II                                     | 10                   | -                        |       |
| III                                    | 3                    | -                        |       |
| Modified Sartorius Score               | 48.6 ± 33.6          | -                        |       |
| IHS4 score, mean ± SD                  | 21.87 ± 15.25        | -                        |       |
| Mild (N)                               | 0                    | -                        |       |
| Moderate (N)                           | 4                    | 11                       |       |
| Severe (N)                             | 11                   | -                        |       |
| Visual Analoge Scale, mean ± SD (range)| 7.53 ± 1.76 (5-10)  | -                        |       |
| Medication use (N)                     | 5 / 10               | -                        |       |
| Systemic retinoids                     | 4                    | -                        |       |
| Adalimumab                             | 1                    | -                        |       |

BMI = body mass index; HS = hidradenitis suppurativa; IHS4 = International hidradenitis suppurativa severity score system; SD = standard deviation, * P < 0.05.

Figure 1. Alpha diversity of the gut microbiome of HS patients and healthy subjects. Shannon index box and whiskers plots showed significantly reduced alpha diversity in HS patients (P = 0.048). HS = hidradenitis suppurativa.
Figure 2. Beta diversity of the gut microbiome of HS patients and healthy subjects. Each data point represents an individual sample. Jaccard Dissimilarity Index showed significantly different microbiome clustering of HS patients and healthy subjects (P = 0.007) presented through principal coordinate Axis 1, indicating that the bacterial community structure in HS is different from that in healthy controls. HS = hidradenitis suppurativa.

Figure 3. Relative abundance of the gut microbiome distribution at the phylum level. The phylum Bacteria_unclassified was significantly reduced (P= 0.032) in the HS group compared to the healthy controls, whereas other phyla showed no significant difference. * P < 0.05 HS = hidradenitis suppurativa.
Coproccocus, Blautia, Ruminococcus2, Fusicatenibacter, Faecalibacterium, Dorea, Firmicutes_unclassified, Dialister, and Ruminococcus were main genera (>1%) in both groups. In HS and control groups, Lachnospiraceae_unclassified (28.85% vs. 27.35%), Ruminococcaceae_unclassified (10.68% vs. 11.54%), Clostridiales_unclassified (7.90% vs. 9.07%), and Roseburia (9.96% vs. 8.66%) were the four commonest genera. Among all genera, the relative abundance of Clostridiales_unclassified, Firmicutes_unclassified, and Fusicatenibacter in HS was significantly lower than in controls (P=0.005, P=0.029, and P=0.046, respectively) (Figure 4).

**Analysis of Fecal Microbiome When Stratified by BMI, Smoking Status, and Treatment of HS Patients**

Shannon and Simpson diversity indices showed no significant differences between non-obese and obese, non-smokers and smokers, and treatment naïve and under treatment groups analyzing alpha-diversity (P=0.536 and P=0.281, P=0.076 and P=0.076, P=0.440 and P=0.254, respectively). Fecal microbiome composition evaluated by Bray-Curtis and Jaccard Dissimilarity indices was not significantly different among non-obese and obese, non-smokers and smokers, and treatment naïve and under treatment groups (P=0.656 and P=0.73, P=0.883 and P=0.729, P=0.391 and P=0.648, respectively). Taxonomic analysis at the phylum and the genus level showed no significant taxa alterations among non-obese and obese, non-smokers and smokers, and treatment naïve and under treatment groups.

**Conclusions**

Based on our hypothesis that the gut microbiome of patients with HS is different from healthy people, we conducted a 16S rRNA-based metagenomics analysis of fecal samples in patients with HS and demonstrated a dysbiotic state in HS patients. We showed significantly decreased alpha diversity of gut microbiome in patients with HS, indicating lower bacterial community richness than healthy controls. In addition, bacterial community structure, beta diversity, was significantly different between patients with HS and healthy controls.

Previous studies on gut microbiome alterations in HS patients are summarized in Table 2 [11-14]. Kam et al and McCarthy et al showed decreased alpha diversity in HS patients in line with our results [12,14]. However, the bacterial community structure of HS patients was different from that of healthy people only in McCarthy et al study [14]. When we compared the bacteria distribution at the phylum level, there was no significant difference other than unclassified bacteria phylum between HS and healthy subjects in our study. On the other hand, our results indicated that the abundance of three genera had been significantly reduced in HS patients: unclassified Clostridiales, Fusicatenibacter, and

![Figure 4](image-url). Relative abundance of the gut microbiome distribution at the genus level. The relative abundance of three genera: unclassified Clostridiales, unclassified Firmicutes, and Fusicatenibacter in HS was significantly lower than that in controls (P = 0.005, P = 0.029, and P = 0.046, respectively). * P < 0.05 HS = hidradenitis suppurativa.
Table 2. Gut microbiome studies conducted with HS patient.

| Study participants       | Ethnicity                              | Current treatment status of participants | Method of the gut microbiome analysis | The minimum time interval between antibiotic usage and sample collection | Alpha-diversity | Beta-diversity | Main findings on the relative abundances of gut bacteria in HS patients in comparison to healthy controls |
|--------------------------|----------------------------------------|------------------------------------------|--------------------------------------|------------------------------------------------------------------------|----------------|---------------|--------------------------------------------------------------------------------------------------------------------------------|
| Present study            | 15 HS vs. 15 HC                        | All Caucasians                           | Fecal samples 16S rRNA               | 3 months                                                               | Decreased alpha-diversity in Shannon index | Significantly different bacterial compositions in Bray-Curtis and Jaccard Dissimilarity Indices | Decreased relative abundance of phylum Unclassified_bacteria | Decreased relative abundance of 3 genera: Clostridiales_unclassified, Firmicutes_unclassified, and Fusisatenibacter |
| Eppinga et al, 2016 [11]| 17 HS vs. 33 HC                        | 76% of HS and HC are Caucasians          | Fecal samples 16S rRNA               | Decreased alpha-diversity in Shannon index | No significant results in Simpson, Faith's phylogenetic diversity, ACE, and Chao indices | Decreased relative abundance of phylum Unclassified_bacteria | Decreased relative abundance of Faecalibacterium prausnitzii or Escherichia coli |
| Kam et al, 2021 [12]    | 3 HS vs. 3 HC                         | African-American: 3 and Hispanic: 0 in HS group African-American: 2 and Hispanic: 1 in the HC group | Fecal samples 16S rRNA               | Decreased alpha-diversity in Shannon index | No significant differences in UniFrac distance matrices | Increased relative abundance of phylum Firmicutes | Increased relative abundance of Bilophila and Holdemania Decreased relative abundance of Lachnobacterium and Veillonella |
| Lam et al, 2021 [13]    | 17 HS vs. 20 HC                        | 13 Caucasians in the HS group and 15 Caucasians in the HC group | Fecal samples 16S rRNA               | 8 weeks and 7 days                                                   | No significant differences in Bray-Curtis and Jaccard Dissimilarity Indices | Increased relative abundance of Robinsoniella, Sellimonas, Eggerthella, Flavonifractor, Oscillibacter, Lachnoclostridium, and Romboutsia | Decreased relative abundance of Faecalibacterium prausnitzii and Escherichia coli |

HC = healthy controls; HS = hidradenitis suppurativa; OUT = operational taxonomic unit; rRNA = ribosomal ribonucleic acid; TNF = tumor necrosis factor.

unclassified Firmicutes. Various taxa alterations have been noted in studies conducted with HS patients, and there was no common bacterial taxa alteration between our study and the other study results (Table 2) [11-14]. These differences between study results in taxa distribution could be attributed to ethnic diversity and consequently different dietary habits of the study samples [17].

Eppinga et al investigated the abundance of two species in the gut microbiome of psoriasis and HS patients with and without concomitant inflammatory bowel diseases (IBD): Faecalibacterium prausnitzii and Escherichia coli. The study reported significantly reduced Faecalibacterium prausnitzii in solely psoriasis patients, psoriasis with IBD patients, and IBD patients. In addition, the study showed a significant reduction in the abundance of Faecalibacterium prausnitzii in patients with concomitant IBD and HS. However, there was no significant difference in Faecalibacterium prausnitzii in patients with solely HS. Compatibly, the abundance of the
The gut microbiome plays a critical role in human health through the development of immune responses mediated by metabolic products and inflammatory signaling pathways [18]. It has been shown that commensal bacteria produce immunomodulatory metabolites, particularly short-chain fatty acids (SCFAs) like butyrate, propionate, and acetate. These metabolites have anti-inflammatory actions mediated by G-protein coupled receptors and contribute to the epithelial barrier integrity [19]. Short-chain fatty acids producing bacteria induce peripheral T-regulatory cells (Tregs), eliminate the Th17/Th1 response and provide a balance between pro-inflammatory and anti-inflammatory immune cells [6,20-23]. Some bacterial taxa are well-characterized with their anti-inflammatory properties. For example, Firmicutes phylum is known for its anti-inflammatory actions via producing SCFAs [18]. Three genera, unclassified Clostridiales, Fusicatenibacter, and unclassified Firmicutes that were shown to be decreased in our study, belong to Firmicutes phylum. Faecalibacterium prausnitzii and Roseburia species are other well-known anti-inflammatory bacteria and play a critical role in IBD [24,25]. The relative abundance of Faecalibacterium and Roseburia was not different between the gut microbiome of patients with HS and healthy controls. However, decreased abundance of Fusicatenibacter was remarkable in HS patients. Fusicatenibacter saccharivorans is a recently isolated and cultured bacterium and a strain of Clostridium subcluster XIVa that induces Tregs and produces butyrate and other SCFAs [26,27]. A recent study has reported that F. saccharivorans decreased in patients with active ulcerative colitis (UC), and there is a negative correlation between the abundance of Fusicatenibacter saccharivorans and UC activity [28]. Therefore, a lower abundance of unclassified Clostridiales, Fusicatenibacter, and unclassified Firmicutes in the gut microbiome of patients with HS may be a triggering factor for systemic inflammation through decreased SCFA production and dysregulation of inflammatory mechanisms, which lead to a shift toward a pro-inflammatory state.

Seven of 15 HS patients included in our study were obese, and 13 out of 15 patients with HS were current smokers. Obesity is a common comorbidity in patients with HS and is considered a risk factor in the development of HS along with smoking and aberrant regulation of innate immunity [15,29]. Gut microbiome alterations may underlie a common pathophysiological process for HS, obesity, smoking, and aberrant immune response by producing various microbial metabolites and consequent inflammation. Recently, several studies in humans and animal models have shown the gut microbiome impact on obesity, and dysbiosis of the gut microbiota is closely associated with obesity [30-32]. A few studies in the literature show the effect of smoking on the gut microbiome. These studies have found an association between smoking status and gut microbiome composition, which is that the composition of the gut microbiome of smokers is different from that of non-smokers [33-35]. In our study, there were no significant differences neither in diversity nor taxa distribution between the gut microbiome of obese and healthy subjects or the gut microbiome of smokers and non-smokers.

Several limitations of this study should be noted. First, we could not perform a power analysis, and the sample size was small in study subgroups. Larger sample size should be concluded with more significant results in future studies. Second, although we excluded participants using antibiotics, probiotics, prebiotics, and on a specific diet, not following a standard diet among participants may have affected the composition of the gut microbiome. Third, there were five HS patients under treatment during fecal sample collection in our study. We did not find significant differences in diversity and bacteria distribution between patients under and without treatment. Nevertheless, the inclusion of patients with similar treatment status is warranted to understand better the gut microbiome’s role in the pathogenesis of HS.

This study indicated that significant alterations in the gut microbiome of HS patients could play a critical role in the pathogenesis of HS. These findings also provide further insights that the gut–skin axis contributes to the pathogenesis of chronic inflammatory skin disorders. Further investigations are required to explain the connections between the gut microbiome and the pathogenesis of HS. Increased understanding of the pathogenesis of HS will shed light on the new potential therapeutic targets, and novel treatment options such as probiotic and prebiotic supplementation or even fecal microbiome transplantation will arise in the management of this challenging chronic skin disorder.

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