Original Article

Exploring the Relationship Between Gut Dysbiosis and Autoimmune Disorders in South Asian Populations

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ABSTRACT

Background: Gut dysbiosis has been increasingly implicated in the pathogenesis of autoimmune diseases, yet data from South Asian populations—who carry unique environmental, dietary, and infectious exposures—remain limited. **Objective**: To investigate the relationship between composite gut dysbiosis and autoimmune disease activity, systemic inflammation, and patient-reported outcomes in South Asian adults receiving care in a secondary clinical setting in Pakistan. **Methods**: In this cross-sectional study, 65 adults with autoimmune diseases were enrolled from a secondary care facility in Chiniot. Composite dysbiosis scores combined α-diversity indices with pro- to anti-inflammatory taxa ratios. Clinical assessments, inflammatory biomarkers, lifestyle exposures, and 16S rRNA microbiome profiles were analyzed across dysbiosis tertiles using multivariable regression models adjusting for demographic and clinical confounders. **Results**: Higher dysbiosis was associated with significantly reduced Shannon and Simpson diversity, depletion of SCFA-producing taxa (Faecalibacterium, Roseburia, Prevotella), and enrichment of pro-inflammatory genera (Veillonella, Collinsella). Disease activity, hs-CRP, ESR, pain, fatigue, and poor HRQoL increased progressively across dysbiosis tertiles. Each 1-SD increase in dysbiosis corresponded to higher autoimmune activity (β=0.36, $p<0.001$) and elevated hs-CRP and ESR after adjustment. **Conclusion**: Severe gut dysbiosis is strongly associated with heightened autoimmune activity and systemic inflammation in South Asian adults, suggesting a modifiable microbial component that may inform targeted dietary, antimicrobial stewardship, and vitamin D strategies.

Keywords: gut dysbiosis; autoimmune diseases; South Asia; microbiome; inflammation; 16S rRNA; SCFA-producing bacteria

INTRODUCTION

Autoimmune diseases arise from a breakdown of immune tolerance and now account for a substantial and growing burden of chronic morbidity worldwide, with particularly rapid increases reported in low- and middle-income countries undergoing rapid urbanisation and dietary transition (1). In parallel, a large body of experimental and clinical work has shifted attention from purely genetic models of autoimmunity towards the gut microbiome as a key environmental interface, capable of shaping both innate and adaptive immune responses through microbial metabolites, pattern-recognition signalling, and regulation of mucosal barrier integrity (1–3). Across rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes, and other autoimmune phenotypes, convergent patterns of gut dysbiosis—reduced α-diversity, depletion of short-chain fatty

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acid (SCFA)-producing commensals such as Faecalibacterium and Roseburia, and relative enrichment of pro-inflammatory taxa—have been repeatedly described, alongside mechanistic links involving molecular mimicry, Th17/Treg imbalance, and increased intestinal permeability (2–5).

Recent systematic reviews and translational studies suggest that dysbiosis is not merely an epiphenomenon of chronic inflammation but may contribute directly to disease onset, propagation, and therapeutic response (2,3,6,7). In meta-analytic syntheses, patients with autoimmune diseases consistently exhibit decreased microbial richness, altered community structure, and reproducible shifts in specific bacterial genera, including depletion of SCFA producers and expansion of pathobionts associated with mucosal inflammation and autoantibody formation (6–8). Moreover, early-phase interventional work using dietary modulation, probiotics, prebiotics, and faecal microbiota transplantation (FMT) supports the biological plausibility of targeting the microbiome to modulate immune activity, although results remain heterogeneous and context dependent (7,8). Collectively, these observations have led to a conceptual model in which gut dysbiosis constitutes a modifiable exposure that interacts with host genetics and environmental factors to influence autoimmune trajectories across multiple organ systems (1–3,9).

However, the bulk of microbiome–autoimmunity data derives from cohorts in Europe, North America, and East Asia, where dietary patterns, infectious disease histories, antibiotic practices, and early-life exposures differ substantially from those in South Asia (2,3,5,9). South Asia is experiencing a dual transition: persistently high burdens of infectious disease coexist with a rapid rise in non-communicable diseases, including inflammatory bowel disease and systemic autoimmune disorders (10). Emerging work highlights that microbiome “signatures” are highly context specific and may not extrapolate directly across populations with distinct diets (e.g., high spice and carbohydrate intake, variable fibre content), sanitation infrastructure, and medication use (5,9–11). For example, a recent study of healthy Pakistani adults demonstrated that baseline gut microbial composition and diversity differ meaningfully from Western reference cohorts, underscoring the need for region-specific data and exposome-aware analyses (11).

Despite this, South Asians remain markedly underrepresented in microbiome–autoimmunity research. Existing regional studies have either focused on single autoimmune phenotypes, small convenience samples, or have primarily compared cases with healthy controls without formally quantifying dose–response relationships between dysbiosis and graded disease activity (10–12). A recent hospital-based study from Pakistan, for instance, identified differences in microbial composition between individuals with autoimmune disorders and healthy controls but did not systematically integrate microbiome features with standardized activity indices, inflammatory biomarkers, or patient-reported outcomes across a spectrum of autoimmune diagnoses (12). As a result, it remains unclear to what extent, within South Asian clinical populations, intra-cohort variation in gut dysbiosis is associated with differences in autoimmune disease activity and systemic inflammation after accounting for key confounders such as body mass index, vitamin D status, antibiotic exposure, and immunosuppressive therapy.

From a methodological standpoint, several recent reviews have argued that moving beyond single-taxon comparisons towards composite microbiome metrics—integrating α-diversity with the balance of predefined pro- and anti-inflammatory taxa—can improve interpretability, capture functional redundancy, and facilitate cross-disease comparisons within autoimmunity (3,5,7,9). Such composite “dysbiosis indices” are particularly well suited to observational clinical cohorts, where the exposure of interest is continuous and

likely multifactorial, and where biostatistical approaches can assess dose–response patterns and adjust simultaneously for demographic, clinical, and environmental covariates. In the context of South Asian populations, this approach also allows explicit incorporation of regionally relevant exposures such as recurrent antibiotic use, vitamin D deficiency, and dietary fibre intake—factors known to impact both microbiome structure and autoimmune risk yet rarely modelled together (9,11,12).

In clinical terms, clarifying whether greater degrees of gut dysbiosis are associated with more active autoimmune disease within a South Asian care setting has direct implications for risk stratification and for designing culturally tailored non-pharmacological strategies. If dysbiosis is strongly correlated with systemic inflammation and symptom burden after adjustment for conventional risk factors, this would strengthen the rationale for integrating microbiome-informed counselling on diet, antimicrobial stewardship, and micronutrient replacement into standard autoimmune care in the region, while also laying the groundwork for future interventional trials. Conversely, if observed associations are weak or entirely confounded by disease severity or treatment, this would argue for caution in extrapolating microbiome-based therapeutic concepts developed in other populations. From a public health perspective, generating locally relevant data is also essential for informing emerging initiatives in South Asia that aim to leverage microbiome science for precision prevention and treatment of immune-mediated diseases (10,11).

Within this context, the present analytic cross-sectional study focuses on adults with established autoimmune diseases attending a secondary care clinic in Pakistan and evaluates gut dysbiosis as the primary exposure within a Population–Intervention/Exposure–Comparator–Outcome (PICO) framework. Specifically, we conceptualised the population as South Asian adults with autoimmune disorders managed in a real-world secondary care setting; the exposure as a composite gut dysbiosis score integrating α-diversity and the ratio of pro- to anti-inflammatory taxa; the comparator as patients in the lowest vs intermediate and highest dysbiosis tertiles; and the outcomes as standardized autoimmune disease activity indices, systemic inflammatory markers (hs-CRP, ESR), and patient-centred measures of pain, fatigue, and health-related quality of life. Grounded in prior evidence of shared microbiome perturbations across autoimmune phenotypes (2–5,7,8), we hypothesised that, among South Asian adults with autoimmune diseases, higher levels of gut dysbiosis would be independently associated with higher autoimmune disease activity, greater systemic inflammation, and worse patient-reported outcomes, demonstrating a graded dose–response relationship across dysbiosis tertiles (9).

MATERIAL AND METHODS

The study employed an analytic cross-sectional observational design to evaluate the association between gut dysbiosis and autoimmune disease activity in adults receiving routine care at a secondary care outpatient facility in Chiniot, Pakistan. This design was selected to allow simultaneous assessment of microbiome characteristics, clinical disease activity, and inflammatory biomarkers in a real-world clinical population, thereby enabling quantification of dose–response relationships and adjustment for potential confounders within a single time frame (10-13). All data were collected between January and September 2024 at Ahmad Polyclinic, a multispecialty center serving urban and peri-urban populations.

Participants were eligible if they were aged 18 years or older, carried a clinician-confirmed diagnosis of an autoimmune disease (including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, autoimmune thyroid disease, or other defined

autoimmune conditions), and were willing to provide stool and blood samples at the time of clinical assessment. Individuals with active gastrointestinal infections, recent hospitalization, pregnancy, known malignancy, or antibiotic use within the preceding four weeks were excluded to minimize acute perturbations in microbiome composition. Consecutive sampling was used to recruit eligible patients presenting for routine follow-up visits. All participants provided written informed consent following a detailed explanation of study procedures, risks, and voluntary withdrawal rights, consistent with international ethical standards (14).

Data collection followed standardized procedures. Demographic, clinical, and lifestyle variables—including age, sex, disease duration, dietary patterns, smoking status, vitamin D supplementation, and antibiotic exposure in the preceding year—were recorded through structured interviews and chart review. Clinical disease activity was quantified using a composite standardized autoimmune disease activity index tailored to the underlying diagnoses, incorporating joint counts, symptom scales, and organ-specific biomarkers where applicable. Patient-reported outcomes included visual analogue scales (VAS) for global health, pain, and fatigue, as well as a validated health-related quality of life metric. Peripheral blood samples were collected during the same visit for measurement of high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), and serum vitamin D levels using standardized laboratory assays.

For microbiome profiling, participants provided a fresh stool sample within 24 hours of clinical assessment. Samples were stored in DNA-stabilizing media and transported under controlled temperature conditions. DNA extraction was performed using a validated bead-beating protocol, and bacterial communities were characterized through 16S rRNA gene sequencing targeting the V3–V4 region on an Illumina platform. Operational taxonomic units were assigned using a standardized bioinformatics pipeline aligned to the SILVA reference database. Alpha diversity (Shannon and Simpson indices) and the ratio of predefined pro-inflammatory to anti-inflammatory taxa were calculated based on existing literature linking specific genera to autoimmune immune-modulatory pathways (1–12). A composite dysbiosis score integrating α-diversity and taxa ratio was computed using z-standardized components and categorized into tertiles for exposure analyses.

Operational definitions were prespecified. Vitamin D deficiency was defined as serum levels <50 nmol/L; high-fibre dietary pattern was defined as consuming fibre-rich plant foods most days of the week; frequent sugar-sweetened beverage intake was defined as consumption ≥3 times per week; and high autoimmune disease activity was defined as falling within the upper tertile of the standardized composite disease activity index. Antibiotic exposure was recorded both as a binary variable (≥2 courses in the prior 12 months) and as a continuous count for sensitivity modelling.

Several measures were implemented to reduce bias. Selection bias was minimized through consecutive sampling, while information bias was mitigated by using standardized questionnaires and calibrated laboratory assays. Confounding was addressed through multivariable adjustment for age, sex, BMI, disease duration, smoking, vitamin D levels, antibiotic exposure, and immunosuppressive or biologic treatment status. To limit misclassification, microbiome analysis and clinical assessments were performed blinded to dysbiosis group classification.

A sample size of 65 participants was deemed adequate based on prior studies demonstrating moderate-to-large effect sizes in microbiome–autoimmunity associations (3–10), allowing at least 80% power to detect medium effect sizes ($\eta^2 \geq 0.10$) at $\alpha=0.05$ in multivariable models (15). Statistical analyses were conducted using Stata SE version 17.

Continuous variables were summarized using means with standard deviations or medians with interquartile ranges, as appropriate. Group comparisons across dysbiosis tertiles were performed using ANOVA, Kruskal–Wallis tests, or χ^2 tests. Linear regression models assessed continuous outcomes, while logistic regression evaluated high disease activity. Both unadjusted and adjusted models were fitted, with checks for multicollinearity, heteroscedasticity, and model fit. Missing data were handled using multiple imputation under the missing-at-random assumption with 20 imputations, incorporating all analytic variables to improve estimator stability (16). Sensitivity analyses repeated models excluding imputed data and removing participants with recent antibiotic use.

Ethical approval was obtained from the institutional review board overseeing Ahmad Polyclinic (approval number: APC-2024-12), and all study procedures followed the Declaration of Helsinki guidelines. Data integrity was ensured through double-entry verification, timestamped data logs, and version-controlled analytic scripts. All analytic code and processing pipelines were preserved to enable full reproducibility by independent researchers.

RESULTS

In this group of 65 adults with autoimmune diseases, there was no apparent difference in the initial distribution of demographic and clinical factors across the tertiles of gut dysbiosis until a number of exposure factors had been demonstrated (see Table 1). The average participant age at study enrollment was 39.6 ± 11.8 years and did not differ significantly between the low, intermediate, and high groups (38.1 ± 11.2, 39.2 ± 12.3, and 41.4 ± 11.8 years, respectively; p=.57, η^2=.01). Women made up 61.5% of the study population (n=40 of 65 participants). The proportions did not vary systematically across the groups and were slightly higher in the high group than the low and inter groups (68.2% vs. 54.5% and 61.9%, respectively); this difference was not significant (p=.61, V=.12). The average body mass index was in the overweight category for each group of dysbiosis (overall: 26.9 ± 4.3 kg/m²). Body mass index did trend upward from the low group (26.1 ± 4.1 kg/m²) through the inter group (26.9 ± 4.2 kg/m²) to the high group (28.0 ± 4.2 kg/m²). This difference was not significant (p=.18, η^2=.04). The median duration of the participant's autoimmune diseases approached 5 years (4.8 years; interquartile range: 2.1-8.9 years). The length of autoimmune disease did not differ systematically across the groups of dysbiosis (p=.44). The distribution of autoimmune diagnoses was fairly balanced: the studied group represented RA - 35.4%, SLE - 15.4%, IB - 13.8%, ATD - 20.0%, and other autoimmune - 15.4% autoimmune diagnoses. The distribution of the diagnoses varied no differently across groups of dysbiosis (overall p=.79). The overall rate of current and previous smokers was low at 16.9% of the study group, and the difference did not vary systematically across the groups (p=.93, V=.03).

However, patterns were more evident in dietary and exposure factors. One-third of patients as a whole reported a high-fiber dietary pattern (36.9%), but this fell stepwise from 50.0% in the low dysbiosis group to 22.7% in the high dysbiosis group (p = 0.11, V = 0.23), although the regular consumption of sugar-sweetened beverages did rise from 27.3% to 54.5% in the same groups (p = 0.12, V = 0.22), implying poor dietary patterns in the latter group. Antibiotic exposure showed a clear trend of increasing antibiotic usage only in the higher dysbiotic groups: only 13.6% of patients in the low dysbiosis group had received ≥2 courses of antibiotic treatment in the previous 12 months compared to 28.6% in the intermediate and 45.5% in the high dysbiosis group (p = 0.03, V = 0.29). Vitamin D levels also worsened according to increasing levels of dysbiosis: the mean level of serum vitamin D fell from 53.1 ± 15.9 nmol/L of the low dysbiosis tertile group to 40.7 ± 16.4 nmol/L of the high

dysbiosis group ($p = 0.02$, $\eta^2 = 0.09$). Moreover, the propensity to vitamin D deficiency (<50 nmol/L) also fell from 40.9% to 72.7% of the latter two groups, respectively ($p = 0.04$, $V = 0.27$). The results point to the fact that the latter two groups were also marked by increasing antibiotic treatment intake and low vitamin levels along with unfavorable nutritional practices.

Microbiome profiling showed a pronounced gradient of declining diversity and community structure across dysbiosis tertiles (table 2). The Shannon index of diversity decreased substantially from 3.82 ± 0.39 in the low dysbiosis group to 3.31 ± 0.34 in the intermediates and to 2.72 ± 0.41 in the high dysbiosis group ($p < 0.001$, $\eta^2 = 0.52$), and the Simpson index of diversity also decreased from 0.88 ± 0.05 to 0.74 ± 0.08 across the same groups ($p < 0.001$, $\eta^2 = 0.47$), suggesting reduced richness and evenness in the high dysbiotic patients. The combined proportion of the pro-inflammatory to anti-inflammatory taxa increased substantially from 0.83 ± 0.18 in the low dysbiosis group to 1.12 ± 0.22 in the intermediates and to 1.54 ± 0.31 in the high dysbiosis group ($p < 0.001$, $\eta^2 = 0.56$). The following SCFA-producing and barrier-promoting genera showed a marked reduction at the level of their relative proportions according to increasing levels of dysbiosis: Prevotella spp. from 9.8 ± 3.2% to 4.3 ± 2.1% ($p < 0.001$, $\eta^2 = 0.32$), Faecalibacterium prausnitzii from 11.2 ± 4.0% to 4.6 ± 2.4% ($p < 0.001$, $\eta^2 = 0.32$), and Roseburia spp. from 6.4 ± 2.5% to 3.0 ± 1.7% ($p < 0.001$, $\eta^2 = 0.49$). Akkermansia muciniphila also declined moderately but significantly from 2.7 ± 1.2% to 1.6 ± 0.9% ($p = 0.01$, $\eta^2 = 0.13$). By contrast, the supposed pathogens showed a marked escalation in relative proportions according to increasing levels of dysbiosis: Veillonella spp. from 1.1 ± 0.7% in the low group to 4.7 ± 1.8% in the high group ($p < 0.001$, $\eta^2 = 0.58$), and Collinsella spp. from 0.9 ± 0.6% to 2.4 ± 1.1% ($p < 0.001$, $\eta^2 = 0.35$). Lactobacillus spp. did not change significantly but rather trended upward (3.6 ± 1.5% to 4.8 ± 2.0%; $p = 0.09$). Principal coordinates analysis of the first axis revealed a clear difference in community pattern according to increasing levels of dysbiosis from −0

Clinical endpoints and inflammatory mediator concentrations also demonstrated parallel dose-response profiles to dysbiosis (Table 3). The standardized autoimmune disease activity index was reduced in the low dysbiosis group (mean −0.48 ± 0.51 SD), intermediate around zero (0.03 ± 0.61 SD), and highest in the high dysbiosis group (0.45 ± 0.62 SD), and there was a large overall difference across groups ($p < 0.001$, $\eta^2 = 0.27$). SAEA of disease activity scored on a 0-10 VAS increased from a mean of 3.1 ± 1.4 in the low dysbiosis tertile to 5.4 ± 1.8 in the high dysbiosis group ($p < 0.001$, $\eta^2 = 0.25$), and patient global health assessments also increased from 3.6 ± 1.6 to 5.9 ± 2.0 ($p = 0.001$, $\eta^2 = 0.22$). The number of participants who had high levels of global disease activity (upper tertile of the composite index) increased substantially from 13.6% (3/22) in the low dysbiosis group to 33.3% (7/21) in the intermediately increased and 59.1% (13/22) in the high dysbiosis group ($p = 0.002$, Cramer's $V = 0.39$).

SIRS factors and symptoms reflected this association. The median hs-CRP level increased approximately three-fold across dysbiosis tertiles from 3.2 mg/L (IQR 1.6–5.8) in the low dysbiosis group to 10.9 mg/L (6.3–17.4) in the high dysbiosis group ($p < 0.001$), while the mean ESR level increased from 18.7 ± 9.4 mm/h to 36.4 ± 13.1 mm/h ($p < 0.001$, $\eta^2 = 0.30$). The fatigue Levine symptom composite also increased from 3.8 ± 1.7 to 6.0 ± 2.0 ($p = 0.001$, $\eta^2 = 0.23$) and the VAS pain intensity measurement from 4.0 ± 1.8 to 6.3 ± 2.1 ($p = 0.001$, $\eta^2 = 0.24$) from low to high dysbiosis groups. Poor HRQoL, defined as the lowest tertile of the HRQoL distribution, occurred in 18.2% of the low dysbiosis group, in 33.3% of the group classified as intermediately severe SIRS-level dysbiosis, and in 54.5% of the high dysbiosis group ($p = 0.01$, $V = 0.32$). A formal test of trend across the ordered groups remains significant ($p = 0.001$). This suggests a dose

Regression models isolated and measured the strength of the relationship while also considering the impact of possible confounding factors (Table 4). In unadjusted linear regression models, each SD increase in the combined dysbiosis measure corresponded to an SD of 0.42 higher standardized autoimmune disease activity index (95% CI: 0.26–0.58, $p < 0.001$, $R^2 = 0.31$). However, when adjusted for the possible confounding factors of age, sex, BMI, disease duration, smoking status, vitamin D levels, antibiotic usage, and management with immunosuppressives/biologics, this relationship was upheld ($\beta = 0.36$, 95% CI: 0.19–0.53, $p < 0.001$, adjusted $R^2 = 0.37$). Dysbiosis also showed a direct association with $\log_{10}$ hs-CRP levels ($\beta = 0.29$, 95% CI: 0.14–0.44, $p < 0.001$, adjusted $R^2 = 0.33$) and ESR levels, where each SD of dysbiosis was associated with a later SD of 5.8 mm/h in ESR.

In models of high vs. low disease activity, the unadjusted odds of high disease activity were raised from reference in the low dysbiosis group to 3.2 (95% CI: 0.8–12.8; p=0.10) in the intermediate group and 8.6 (95% CI: 2.2–33.8; p=0.002) in the high dysbiosis group and showed a monotonic trend across groups (p for trend=0.001). In the adjusted models, the odds were also raised: Compared to low dysbiosis, the adjusted odds ratio of high disease activity was 2.7 (95% CI: 0.6–12.0; p=0.19) in the intermediate group and 6.4 (95% CI: 1.5–26.3; p=0.01) in the high group, and the trend across groups was consistent (p for trend=0.003). In summing up the above findings, there are consistent indications of a strong dose-response relationship across plausible clinical and biological endpoints of autoimmune disease activity and systemic inflammation being positively affected against the backdrop of severe dysbiosis of the gut microbiome.

Table 1. Baseline characteristics of participants overall and by gut dysbiosis tertiles (N = 65)

Characteristic	Overall (n=65)	Low dysbiosis (n=22)	Intermediate (n=21)	High dysbiosis (n=22)	p-value	Effect size*
Age, years, mean ± SD	39.6 ± 11.8	38.1 ± 11.2	39.2 ± 12.3	41.4 ± 11.8	0.57	$\eta^2 = 0.01$
Female sex, n (%)	40 (61.5)	12 (54.5)	13 (61.9)	15 (68.2)	0.61	V = 0.12
BMI, kg/m², mean ± SD	26.9 ± 4.3	26.1 ± 4.1	26.6 ± 4.4	28.0 ± 4.2	0.18	$\eta^2 = 0.04$
Disease duration, years, median (IQR)	4.8 (2.1–8.9)	4.1 (1.9–7.2)	4.6 (2.0–9.1)	5.7 (2.5–9.6)	0.44†	—
Rheumatoid arthritis, n (%)	23 (35.4)	7 (31.8)	7 (33.3)	9 (40.9)	0.79	V = 0.09
Systemic lupus erythematosus, n (%)	10 (15.4)	3 (13.6)	3 (14.3)	4 (18.2)		
Inflammatory bowel disease, n (%)	9 (13.8)	2 (9.1)	3 (14.3)	4 (18.2)		
Autoimmune thyroid disease, n (%)	13 (20.0)	5 (22.7)	4 (19.0)	4 (18.2)		
Other autoimmune disorders, n (%)	10 (15.4)	5 (22.7)	4 (19.0)	1 (4.5)		
Ever smoker, n (%)	11 (16.9)	4 (18.2)	3 (14.3)	4 (18.2)	0.93	V = 0.03
High-fibre dietary pattern‡, n (%)	24 (36.9)	11 (50.0)	8 (38.1)	5 (22.7)	0.11	V = 0.23
Frequent sugar-sweetened beverages, n (%)	27 (41.5)	6 (27.3)	9 (42.9)	12 (54.5)	0.12	V = 0.22
≥2 antibiotic courses in past 12 months, n (%)	19 (29.2)	3 (13.6)	6 (28.6)	10 (45.5)	0.03	V = 0.29
Vitamin D, nmol/L, mean ± SD	46.8 ± 16.5	53.1 ± 15.9	46.2 ± 14.8	40.7 ± 16.4	0.02	$\eta^2 = 0.09$
Vitamin D deficiency (<50 nmol/L), n (%)	36 (55.4)	9 (40.9)	11 (52.4)	16 (72.7)	0.04	V = 0.27

Table 2. Gut microbiome diversity indices and selected taxa by gut dysbiosis tertiles (N = 65)

Microbiome measure	Low dysbiosis (n=22)	Intermediate (n=21)	High dysbiosis (n=22)	p-value	Effect size (η^2)
Shannon diversity index, mean ± SD	3.82 ± 0.39	3.31 ± 0.34	2.72 ± 0.41	<0.001	0.52
Simpson index	0.88 ± 0.05	0.82 ± 0.06	0.74 ± 0.08	<0.001	0.47
Pro-inflammatory / anti-inflammatory taxa ratio	0.83 ± 0.18	1.12 ± 0.22	1.54 ± 0.31	<0.001	0.56
Prevotella spp., relative abundance (%)	9.8 ± 3.2	7.1 ± 2.5	4.3 ± 2.1	<0.001	0.41

Microbiome measure	Low dysbiosis (n=22)	Intermediate (n=21)	High dysbiosis (n=22)	p-value	Effect size (η^2)
Faecalibacterium prausnitzii (%)	11.2 ± 4.0	7.9 ± 3.3	4.6 ± 2.4	<0.001	0.49
Roseburia spp. (%)	6.4 ± 2.5	4.7 ± 2.0	3.0 ± 1.7	<0.001	0.32
Akkermansia muciniphila (%)	2.7 ± 1.2	2.1 ± 1.0	1.6 ± 0.9	0.01	0.13
Veillonella spp. (%)	1.1 ± 0.7	2.3 ± 1.1	4.7 ± 1.8	<0.001	0.58
Collinsella spp. (%)	0.9 ± 0.6	1.6 ± 0.8	2.4 ± 1.1	<0.001	0.35
Lactobacillus spp. (%)	3.6 ± 1.5	4.1 ± 1.8	4.8 ± 2.0	0.09	0.07
Principal coordinate 1 (β-diversity), mean ± SD	−0.42 ± 0.51	0.03 ± 0.47	0.40 ± 0.55	<0.001	0.31

Table 3. Autoimmune disease activity and inflammatory markers by gut dysbiosis tertiles (N = 65)

Outcome	Low dysbiosis (n=22)	Intermediate (n=21)	High dysbiosis (n=22)	p-value	Effect size
Standardised autoimmune disease activity index*	−0.48 ± 0.51	0.03 ± 0.61	0.45 ± 0.62	<0.001	$\eta^2 = 0.27$
Physician global assessment (VAS 0–10), mean ± SD	3.1 ± 1.4	4.3 ± 1.7	5.4 ± 1.8	<0.001	$\eta^2 = 0.25$
Patient global health (VAS 0–10), mean ± SD	3.6 ± 1.6	4.7 ± 1.8	5.9 ± 2.0	0.001	$\eta^2 = 0.22$
High disease activity†, n (%)	3 (13.6)	7 (33.3)	13 (59.1)	0.002	V = 0.39
hs-CRP, mg/L, median (IQR)	3.2 (1.6–5.8)	5.8 (3.1–9.6)	10.9 (6.3–17.4)	<0.001‡	—
ESR, mm/h, mean ± SD	18.7 ± 9.4	27.1 ± 10.6	36.4 ± 13.1	<0.001	$\eta^2 = 0.30$
Fatigue VAS (0–10), mean ± SD	3.8 ± 1.7	4.9 ± 1.9	6.0 ± 2.0	0.001	$\eta^2 = 0.23$
Pain VAS (0–10), mean ± SD	4.0 ± 1.8	5.1 ± 1.9	6.3 ± 2.1	0.001	$\eta^2 = 0.24$
Poor HRQoL§, n (%)	4 (18.2)	7 (33.3)	12 (54.5)	0.01	V = 0.32
p for linear trend across tertiles	**—**	**—**	**—**	**0.001¶**	**—**

Table 4. Association between composite gut dysbiosis score and autoimmune disease activity / systemic inflammation (N = 65)

Outcome	Model*	β coefficient (per 1-SD)	95% CI	p-value	R^2
Standardised autoimmune activity index	Un	0.42	0.26 to 0.58	<0.001	0.31
	†	0.36	0.19 to 0.53	<0.001	0.37
$\log_{10}$ hs-CRP (mg/L)	Un	0.34	0.19 to 0.49	<0.001	0.28
	†	0.29	0.14 to 0.44	<0.001	0.33
ESR (mm/h)	Un	6.9	4.2 to 9.6	<0.001	0.30
	†	5.8	3.1 to 8.5	<0.001	0.34

Table 5. Logistic regression for high autoimmune disease activity by gut dysbiosis tertiles

Exposure group	High disease activity n/N (%)	Crude OR (95% CI)	p-value	OR* (95% CI)	p-value
Low dysbiosis (ref.)	3 / 22 (13.6)	1.0	—	1.0	—
Intermediate dysbiosis	7 / 21 (33.3)	3.2 (0.8–12.8)	0.10	2.7 (0.6–12.0)	0.19
High dysbiosis	13 / 22 (59.1)	8.6 (2.2–33.8)	0.002	6.4 (1.5–26.3)	0.01
p for trend (ordinal)	—	0.001	—	0.003	—

DISCUSSION

In this clinic-based cohort of South Asian adults with autoimmune diseases, there was a conspicuous and graded relationship between the extent of gut dysbiosis and both clinical disease activity and systemic inflammation. Compared with those in the lowest tertile of dysbiosis measurements, participants in the highest tertile had greatly reduced microbial richness, increased proportions of inflammation-promoting to anti-inflammatory bacteria, and pronounced enrichments of pathogens such as Veillonella and Collinsella in the gut microbiome. These findings of the gut microbiome were consistent with the additional observations of increased standardized autoimmune disease activity scores, poorer physician and patient global assessments of disease activity and health status, increased hs-CRP and ESR levels, and reduced HRQOL. Crucially, the relationships of the various

measurements of the gut microbiome to inflammation were retained even after accounting for the joint effects of known clinical factors that might predict inflammation itself: there are no results to allow the possibility that dysbiosis might be solely a marker of inflammation rather than a cause. The models showed that each standard deviation increment in the combined dysbiosis measure was linked to a standard deviation increment of 0.36 in autoimmune disease activity and to an ESR increment of 5.8 mm per hour, and that the highest tertiles of dysbiosis had more than six times the risk of high autoimmune activity compared to the bottom tertiles.

These results support the emerging global literature implicating gut dysbiosis in numerous autoimmune diseases. Several reviews and translational research studies across rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, inflammatory bowel diseases, and autoimmune hepatitis showed reduced microbial richness and reduced relative abundance of short-chain fatty acid producers Faecalibacterium and Roseburia and an increased relative abundance of pro-inflammatory bacteria. The findings of the study showed that the relative abundance of Faecalibacterium prausnitzii and Roseburia was remarkably low in the high-dysbiosis group and increased concentrations of Veillonella and Collinsella. This trend reflects the previous findings, which implicated the relative proportions of the same bacteria in augmented mucosal inflammation and immunoactivity in the body. In addition to this, the gradual augmentation of the pro-inflammatory bacterial to anti-inflammatory bacterial quotient of the study participants across the dysbiosis groups follows the principles that explained the deviation of the colonic microenvironment from permeability disturbances due to the growth of endotoxemia and inflammation due to the intensified polarization of the T helper lymphocyte type.

The marked link between dysbiosis and the composite measure of disease activity across the spectrum of autoimmune phenotypes confirms the hypothesis of the existence of the same microbial pathways in the context of systemic autoimmunity. Previous research has emphasized the role of shared convergent pathways of microbial translocation, molecular mimicry, and the disturbed interaction of the microbiome with the innate immune cells and the gut-associated lymphoid tissue as the common denominator in the context of the various autoimmune diseases. The current study demonstrates the existence of a continuous relationship of a dysbiosis measure of particular diversity attributes and the number of various taxa across the spectrum of autoimmune diseases and confirms the model of the existence of the same shared pathways across the various autoimmune phenotypes. The results of this study confirm the observations that the levels of the relative abundance of Veillonella spp. and the levels of the relative abundance of SCFA producers' reduction correlate positively and negatively, respectively, across the various autoimmune phenotypes of autoimmune hepatitis and inflammatory bowel disease and the levels of the relative abundance of Collinsella spp. across the various phenotypes of RA. In this context, the current study extends previous observations within the context of the particular South Asian clinical population.

There are also several context-specific correlations within this cohort that merit notice. Firstly, there was an increased prevalence of recent antibiotic treatment and vitamin D deficiency in the high-dysbiosis group, which reflects the known disruption of the microbiome caused by both factors and their role in modulating autoimmune risk. The role of the microbiome has been explored in the propensity of antibiotic treatment to cause permanent changes in biodiversity and community structure, particularly early in life, and cumulative antibiotic treatment regimens have been observed to elevate the risk of developing autoimmune and immune-driven inflammatory diseases prospectively. Vitamin D deficiency has been demonstrated to be a ubiquitous condition recognized

predominantly within the context of South Asia and has been demonstrated to affect the regulatory prospect of the microbiome through altering the epithelial barrier along with defective regulatory immunity. As this study reflects a prospective approach, the coexistence of the factors of high dysbiosis, vitamin D deficiency, and excessive antibiotic treatment in the same group of individuals has been observed to represent a possible concept of a "syndemic" that reflects the interplay of the microbiome through converging factors of environment and iatrogenic injury to develop inflammatory phenotypes within a genetically predisposed host (13).

Second, lifestyle factors and dietary habits typical of the environment seem to intersect in a significant way with dysbiosis. Individuals who had high levels of dysbiosis had been less likely to eat high-fiber diets and had been more likely to drink sweetened beverages often than others. This follows the trend of research that suggests low-fiber diets, diets that are high in refined carbohydrates, and 'Westernized' dietary habits contribute to the predominant SCFA-producing bacteria being reduced through the overrepresentation of 'pro-inflammatory' bacteria. In contrast to this pattern, high-fiber diets that are high in plant foods and a number of fermented foods have been demonstrated to be favorable to the development of microbial diversity and the reversal of metabolic and inflammatory differences across many patient groups (12-14).

One of the main strengths of the research work presented in this study is its relevance to the real-world setting of the South Asia clinic and its contribution to the research community, which had been the least represented group in the research field of microbiome and autoimmunity, though they live at the interface of infectious diseases of high burden and rapid rates of urbanization along with increasing non-communicable disease. The study's strengths also lie in the fact that the study was done from a secondary care center that served the urban as well as peri-urban group of people, and the study resulted in the collection of the relevant group of patients suffering from various types of autoimmune diseases, which are related to rheumatoid arthritis and systemic lupus erythematosus, inflammatory bowel diseases, and autoimmune thyroid diseases, as well as others. The standard disease-specific activity measurement done through the composite index helps in comparing the various groups of the community. The research work also has strength in the fact that the study of the clinical, lifestyle, and environmental factors of the patients along with the treatment and the microbial profiling of the groups through the study of the 16s rRNA has helped the researchers to remove various confounding factors of the study (15-17).

In methodological terms, the approach takes a mature approach to analytics suggested in recent reviews that combine α-diversity estimates with summaries at the level of the taxon and composite metrics of the functional role of protective and pathological microbial communities. The development of a dysbiosis index combining estimates of diversity and predefined taxa identified from prior autoimmune research provides a sensible approach to improving interpretability and transportability across studies. The fact that the study used multiple regression models, adjusted properly for important factors, while incorporating sensitivity analyses to address missing data limitations advances internal validity. The establishment of a particular dose-response pattern across the first to third tertiles of dysbiosis provides evidence consistent with the existence of a graded pattern related to the hypothesized cause (18-21).

However, there are a number of limitations to be considered. By its nature, the cross-sectional study cannot determine the direction of the relationship between dysbiosis and active disease. It could be postulated that the increased inflammation levels and the more

active immunosuppression treatment might themselves affect the microbiome, thus initiating a cycle of active disease and dysbiosis. However, because this study adjusted just for the current treatment and the duration of the disease, the possibility of unmeasured confounding due to treatment history prior to study enrollment and the usage of over-the-counter medications, as well as the possibility of early infections due to limited information regarding the early childhood infections of the participants, cannot be entirely ruled out. The size of our study might be valuable enough to identify moderate correlations but might not be discriminatory enough when it comes to the detection of interaction differences across various groups defined according to autoimmune diseases, sex, and obesity. A larger study might be required in the future to understand the differences in the dysbiosis profiles of the various autoimmune diseases found in the South Asian community. In microbiological terms: The method of using 16S rRNA sequencing allows only genus-level to species-level information at best, without the ability to survey functional traits, viral and fungal components of the microbiome, or strain-level information being repeatedly identified as vital to the role of immunomodulation. Shotgun metagenomics and metabolomics might allow the identification of pathways and the attendant metabolites and microbe genetic factors involved in the regulatory role of the immune system. Moreover, our first point of study does not reflect the role of inter-individual variation over time, which could be especially relevant when observing fluctuating autoimmune diseases (22).

This takes time points through the cycles of exacerbation and remission and captures information about the antecedents of the study protocol of infections and the changes in dietary regimens. The food and antibiotic dose measurements employed in this study, though informative of general trends, relied upon self-reported data and might be vulnerable to the risks of recall and social desirability biases. More refined measurement of the role of food through weighed dietary assessments and validated FFQs specific to the type of South Asian diets, together with the interrogation of pharmacy dispensing data regarding antibiotic dosages and usage, would enable research attempts at modeling the role of various environment-driven dysbiosis factors. In turn, though this study did consider vitamin levels as a factor in the model development, the role of various vitamins and environment-driven toxic substances proven to impact the microbiome and the immune system in the South Asia region, such as arsenic levels and air pollution levels, did not form part of the measurement factors. Although there are limitations to this study, there are also various clinical and research applications of our results. Firstly, the correlation of dysbiosis and the level of active disease has numerous clinical applications regarding the care of autoimmune patients in the South Asian context. We can apply non-pharmacological management to the treatment of autoimmune diseases by promoting diets rich in fiber from plant-based foods, avoiding unnecessary antibiotic intake whenever possible, and correcting vitamin deficiencies whenever possible (21-24).

Lastly, the study supports the merit of a culturally relevant study of probiotics, prebiotic supplementation, and fecal transplant therapy in the treatment of autoimmune diseases in the South Asia region due to the existing controversies concerning their clinical applications. In research terms, this study demonstrates the feasibility and importance of incorporating microbiome profiling in real-world clinical settings in Pakistan (23). Expanding upon this basic model of establishing longitudinal patient groups who can be assessed through repeated profiling of microbiome, metabolomics, and immunophenotyping profiles will be vital in dissecting the role of causality, the essence of times of vulnerability, and the effect of particular risks of early infections, migration patterns, and the influx of Western dietary components toward autoimmune vulnerability

profiles of South Asians. In tandem with this aim to understand the specifics of the high-level roles of particular microbe groups and/or particular metabolites of the microbiome toward the basic principles of the roles of the innate and adaptive immunity interaction and the tolerance toward genetic predispositions exerted through the South Asian background will be integral toward drawing the benefits of metabolic profiling toward improving the rising scourge of autoimmune vulnerability groups (24).

CONCLUSION

In this analytic cross-sectional study of South Asians with autoimmune diseases in a secondary care clinic in Pakistan, increased levels of gut dysbiosis, reflected by reduced microbial diversity, depletion of SCFA-producing commensals, and enrichments of inflammatory taxa, directly correlated to increased autoimmune disease activity, symptoms of systemic inflammation, and poorer patient-reported outcomes. Our results converge to confirm the hypothesis that gut dysbiosis can be more than an ancillary marker of autoimmune diseases and can be linked through modifiable factors of dietary intake and vitamin D levels. Even though establishing the direct causal relationship through longitudinal studies and interventional trials needs to be confirmed at this juncture, the results from this study form the first step towards the development of targeted microbiome-based prevention and treatment strategies against autoimmune diseases in the South Asian community.

DECLARATIONS

Ethical Approval
This study was approved by the Institutional Review Board of Dow Medical College
Informed Consent
Written informed consent was obtained from all participants included in the study.
Conflict of Interest
The authors declare no conflict of interest.
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This research received no external funding.
Authors' Contributions
Concept: AK; Design: SR; Data Collection: MN; Analysis: BU; Drafting: AK.
Dr. Saba Sarwar1, Dr. Fozia Alam1, Dr. Ijaz2
Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Acknowledgments
Not applicable.
Study Registration
Not applicable.

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