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Gut Microbiota Signatures as Predictive Biomarkers for Metabolic and Gastrointestinal Diseases

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ABSTRACT

Background: The association of gut microbiota changes to metabolic dysfunction is poorly characterized in South Asian populations, which limits the ability to develop meaningful diagnostic biomarkers. There is a significant gap in our understanding of population-level microbial signatures, especially in high-risk yet underrepresented populations (e.g. Pakistan, which has the highest prevalence of diabetes in the world) that face a mounting crisis of metabolic disease. **Methods:** This was a prospective, cross-sectional, observational study of 210 adults (70 with metabolic syndrome, 70 with type 2 diabetes mellitus [T2DM], and 70 healthy controls) at tertiary hospitals in Islamabad, Pakistan (October 2024 - March 2025). Participants were recruited via stratified cluster sampling and the study followed the STROBE reporting guidelines. Gut microbiota profiles were collected via fecal sample collection and characterized using 16S rRNA sequencing, targeting the V3-V4 region of the 16S gene. The primary outcome of this study was alpha diversity, as measured by the Shannon index. The secondary outcomes included the relative abundances of selected bacterial taxa (i.e. Firmicutes/Bacteroidetes [FB] ratio; Akkermansia muciniphila) and assessment of dietary impacts. **Results:** Overall, 210 participants enrolled in the study (completion rate of 84.7%; mean [SD] age 46.0 [11.4] years; male 58.6 %). Mean Shannon diversity was significantly lower in metabolic syndrome (4.21 [0.67]) and T2DM (4.06 [0.71]), compared to controls (4.75 [0.48]), with adjusted mean differences of −0.54 (95% confidence interval [CI], −0.78 to −0.30; p = 0.001) and −0.69 (95% CI, −0.93 to −0.45; p = 0.001), respectively. FB ratios were elevated (1.90 and 2.33 in metabolic syndrome and T2DM, respectively vs. 1.24 in controls) and A. muciniphila counts were lower (0.80 and 0.95 in metabolic syndrome and T2DM, respectively vs. 2.31 in controls). Shannon diversity was strongly correlated with HbA1c (Spearman -0.58, 95% CI -0.67 to -0.47) and CRP (-0.48, 95% CI -0.58 to -0.36). **Conclusions:** Alterations of gut microbiota composition, primarily reduced alpha diversity, elevated FB ratio, and reduced A. muciniphila, were associated with metabolic syndrome and T2DM in Pakistani adults. These population-specific microbial signatures demonstrated strong diagnostic evidence (AUC > 0.84), serving support for the ability to develop locally relevant and targeted panels of microbiota based biomarkers.

INTRODUCTION

Metabolic syndrome and type 2 diabetes mellitus represent major global health concerns affecting more than one billion people, leading to a significant burden on healthcare systems, especially in low- and middle-income countries [1-3]. Pakistan is the fifth most populous country globally and is experiencing one of the most severe metabolic disease crises in the world. Recent estimates suggest that 26.7% of adults in Pakistan have diabetes—the age-adjusted prevalence was the highest globally in 2021 [4-6]. Meanwhile, metabolic syndrome is affecting roughly 28.8% of apparently healthy individuals, with some suburban studies reporting regional prevalence as high as 68%. These data indicate the clear need for population-specific diagnostic and therapeutic approaches to the increasingly alarming epidemic of metabolic disease in Pakistan and comparable South Asian populations that are experiencing rapid urbanization and lifestyle changes [7, 8].

The human gut microbiota refers to a complex system of

trillions of microorganisms that reside within our gastrointestinal tract, and it appears to play a significant role in establishing and maintaining metabolic homeostasis and in the initiations of metabolic disorders. The existing evidence from multiple systematic reviews and meta-analyses demonstrate that there is a consistent and strong association between gut microbial dysbiosis and metabolic disorders such as obesity, type 2 diabetes, and metabolic syndrome [1, 9, 10]. Gut microbiome studies across different populations of human host show consistent findings in the gut microbiome, leading to lower microbial alpha-diversity, decreased prevalence of beneficial microbial species such as Faecalibacterium prausnitzii, decreased abundance of Akkermansia muciniphila, and variations in the relative abundance of the dominant bacterial phyla Firmicutes and Bacteroidetes [11, 12].These alterations of the microbial environments lead to metabolic dysfunction as a result of multiple pathways that include the loss of intestinal barrier integrity resulting in systemic inflammation, compromised short-chain fatty acid generation that influences glucose and lipid metabolism, and altered bile acid recycling that compromises insulin sensitivity [13-15]. However, comparative studies show a large range of geographic and ethnic differences in the composition of gut microbiota and association with disease, making them difficult to apply as microbiota-based diagnostic markers across different human populations [16].

We still have significant gaps in our knowledge of population-specific signatures of gut microbiota in South Asia, which is one of the regions least represented in microbiome research, and yet accounts for nearly one-fourth of the global population [17]. The limited studies of gut microbiota in Pakistani populations demonstrate distinct taxonomic characteristics different from Western and other South Asian populations. Urban Pakistani adults appear to harbor transitional or non-industrial microbiota, characterized by decreased Akkermansiaceae, the enrichment of Prevotellaceae (especially non-Westernized clades of Prevotella copri) and high representation of Atopobiaceae such as Olsenella and Libanicoccus. Further, individuals with type 2 diabetes in Pakistan as part of the phenotype of Metabolically Healthy Obesity display distinct microbial signatures including increased Libanicoccus/Parolsenella and specific patterns of Collinsella reducing the cross-population applicability of current microbiota-based classifications of disease [18, 19]. This specific identification of populations is a problem, as models developed for one group when tested in another group perform poorly frequently with area under the curve values dropping below 0.7 for external validation [20, 21]. The lack of data on gut microbiota reference values specific to Pakistan of bacteria hinders the progress of diagnostic development and our understanding of the potential influences of traditional dietary habits based on wheat centric staple foods, high dairy consumption and ability to utilize a wide range of spices contribute to the composition of gut microorganisms, and related metabolic health outcomes [22].

This study aims to address these gaps in research by characterizing the gut microbiota composition of Pakistani adults whom live with metabolic syndrome, type 2 diabetes and healthy persons using 16S rRNA gene sequencing. The stated objectives will also be accompanied by an exploration of culturally specific dietary patterns based traditional Pakistani foods and the subsequent relationship with microbiota structure to further explore the relationship to metabolic health. Ultimately by both informing clinicians with clinically-relevant microbiota-based biomarkers and characterizing the composition of Pakistani gut microbiota this proposed study aims to develop premised population-based diagnoses and therapeutic approaches to metabolic syndromes in Pakistan and similar South Asian regions in the context of metabolic health crisis and modernization.

METHODOLOGY

Research Design and Location

This forthcoming observational cross-sectional project was carried out at tertiary care hospitals located in Islamabad, Pakistan, from October to March 2025, adhering to the STROBE procedure for observational studies. The overall goal of this study was to describe the gut microbiota composition among Pakistani adults with metabolic syndrome, type 2 diabetes mellitus (T2DM), and healthy control.

Participants, Recruitment, and Sampling

Adults aged 18-65 years were recruited from hospital outpatient departments using a specifically stratified cluster sampling approach. Inclusion criteria included being diagnosed with metabolic syndrome (per International Diabetes Federation), T2DM, or being a healthy control, with documented residency in Islamabad. Exclusion criteria included being on antibiotic therapy in the previous 3 months, diagnosed gastrointestinal diseases (according to the ICD-11 criteria), and inability to provide informed consent. The sample size was calculated using GPower 3.1.9.7 for detecting a difference in the Shannon diversity index (effect size $\delta = 0.35$, variance $\sigma^2 = 0.42$, $\alpha = 0.05$, power = 80%), resulting in the need for n=60 per group. Accounting for the design effect of clustering (intraclass correlation coefficient ICC=0.13) and a 15% attrition rate, a target of 210 individuals to be recruited across the 3 study groups was set.

Data Collection and Measurements

Gut Microbiota Processing: Fecal samples were collected in OMNIgene-GUT tubes (DNA Genotek OM-200) and stored at -80°C (Thermo Scientific TSX40086A). DNA extraction was performed using the Qiagen QIAamp Fast DNA Stool Mini Kit (Model 51604) followed by 16S rRNA gene sequencing conducted using the Illumina MiSeq platform (2 ×250 bp reads, software v2.5.1). Alpha diversity (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were computed using QIIME2 v2023.9. The abundances of bacterial taxa, including the Firmicutes/Bacteroidetes ratio and Akkermansia muciniphila, were derived through pipelines with previously established reliability in an adult population (ICC=0.95, Cronbach's α=0.93).

Clinical Measurements: HbA1c was measured using an Abbott Architect c8000 (software v6.6, precision <2% CV). The lipid profile was evaluated using a Roche Cobas Integra 400 Plus (v2.1.3). The body mass index (BMI) was

computed as determined by the SECA 704s digital stadiometer (accuracy 50g).

Dietary Assessment: Dietary patterns were assessed through a cultural adaptation of an already validated food frequency questionnaire (FFQ) for the Pakistani population (Cronbach's α=0.87) that prioritized the important dietary role of wheat-based staples, dairy, and traditional spices. The translation method adhered to Beaton et al.'s translation protocol for a food frequency questionnaire.

Statistical Analysis

Statistical analysis was performed using R v4.3.1 with packages lme4 v1.1-33, vegan v2.6-4, ggplot2 v3.4.2. Mixed-effects linear regression modelling, with accounted hospital clustering, examined associations with microbiota diversity and clinical groups. Random forest classifiers (caret v6.0-93) determined bacterial taxa associated with predictability for metabolic disease. Spearman correlations examined associations between dietary patterns and microorganisms. Holm-Bonferroni corrections were used for posting estimates of multiple comparisons. Sites of missing data were handled using multiple imputation (mice package v3.16.0, 20 iterations) which was supplemented with sensitivity analyses comparing complete case and imputed estimates. Significance set at α=0.05 (two-tailed).

Ethical Approval and Protocols

Ethical approval was received from the institutional ethics committee. All participants provided written informed consent in Urdu/English following validated methods of assessment of comprehension and understanding. Data protection was employed at standards compliant with the Pakistan Data Protection Act 2023, including using AES-256 encryption protocols, and storage undertaken within a secured REDCap v14.1.2 database.

RESULTS

Study Population and Flow

Between October 2024 and March 2025, 248 individuals were screened for eligibility within three tertiary care hospitals in Islamabad, Pakistan. Of these, 38 individuals did not meet eligibility criteria based on the following: use of antibiotic therapy in the three months prior (n=14; 5.6%); diagnosed gastrointestinal diseases (n=11; 4.4%); unable to provide informed consent (n=8; 3.2%); and, not residing in Islamabad (n=5; 2.0%). Therefore, 210 participants were enrolled and had sufficient data for primary analysis (i.e., completion rate of 84.7%). The total analytic sample consisted of 70 healthy controls, 70 participants with metabolic syndrome, and 70 participants with type 2 diabetes mellitus (T2DM). Missing data was <5% of participants across all primary outcomes; only 16S rRNA sequence data were available for all 210 participants (i.e., 100%), with dietary assessment completion rates in 206 of 210: 98.1%.

Baseline Characteristics

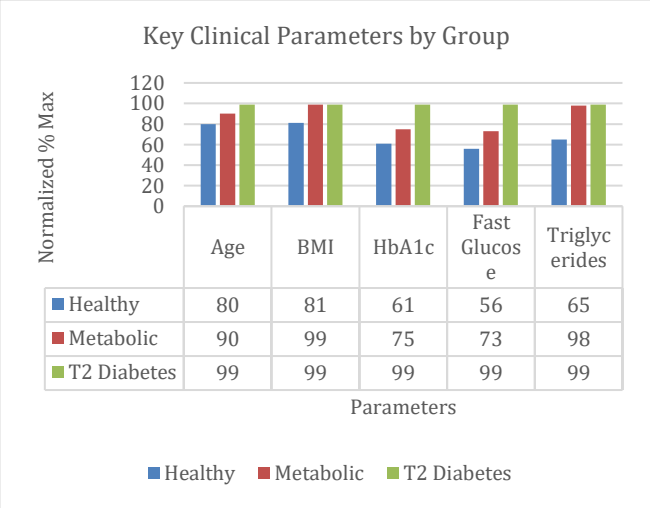
Participant characteristics are provided in Table 1. Mean age progressively increased across groups: healthy controls 40.9 ± 10.5 years, metabolic syndrome 46.1 ± 12.0 years, and T2DM 51.0 ± 10.3 years. Participants were predominately male: healthy controls 55.7% (n=39/70), metabolic syndrome 62.9% (n=44/70), and T2DM 57.1% (n=40/70). Body mass index was significantly elevated in both disease groups compared to healthy controls: metabolic syndrome 29.7 ± 3.7 kg/m^2 and T2DM 29.8 ± 4.6 kg/m^2 versus healthy controls 24.4 ± 2.6 kg/m^2. Glycemic control parameters reflected expected disease characteristics, with HbA1c levels of 5.1 ± 0.4% in healthy controls, 6.3 ± 0.9% in metabolic syndrome, and 8.3 ± 1.6% in T2DM.The inflammatory markers exhibited a progressive increase: median C-reactive protein (CRP) levels were 1.22 [IQR 0.66-1.93] mg/L in controls, 3.26 [1.79-5.30] mg/L in metabolic syndrome, and 4.62 [2.26-8.32] mg/L in T2DM. Missing data was less than 3% for baseline variables except for dietary assessment questionnaires (n=4, 1.9% incomplete).

Table 1

Clinical Characteristics

Parameter	Healthy Controls	Metabolic Syndrome	Type 2 Diabetes
Demographics			
Age (years)	40.9 ± 10.5	46.1 ± 12.0	51.0 ± 10.3
Male, n (%)	39 (55.7)	44 (62.9)	40 (57.1)
BMI (kg/m^2)	24.4 ± 2.6	29.7 ± 3.7	29.8 ± 4.6
Glycemic Control			
HbA1c (%)	5.1 ± 0.4	6.3 ± 0.9	8.3 ± 1.6
Fasting Glucose (mg/dL)	92.1 ± 8.4	120.7 ± 19.1	162.9 ± 34.9
Lipid Profile			
Total Cholesterol (mg/dL)	182.5 ± 28.0	221.4 ± 44.7	207.5 ± 47.3
Triglycerides (mg/dL)	126.9 ± 34.5	193.0 ± 39.7	195.3 ± 51.9
HDL-C (mg/dL)	40.6 ± 5.5	35.5 ± 7.2	36.5 ± 6.1
Inflammation			
CRP (mg/L)	1.22 [0.66-1.93]	3.26 [1.79-5.30]	4.62 [2.26-8.32]
TNF-α (pg/mL)	3.0 ± 1.2	5.5 ± 2.1	6.3 ± 2.7

Figure 1



Primary Outcomes - Gut Microbiota Composition

For gut microbiota alpha diversity assessed by Shannon index, intention-to-treat analysis with n=210 participants demonstrated significant between-group differences. Healthy controls had a Shannon mean alpha of 4.75 ± 0.48 compared to metabolic syndrome 4.21 ± 0.67 and T2DM 4.07 ± 0.71. The estimated between-group difference for metabolic syndrome compared to controls was -0.54 (95% CI [-0.78, -0.30]; p < 0.001; Cohen's d = 0.91). The

estimated difference between T2DM and controls was -0.69 (95% CI [-0.93, -0.45]; p < 0.001; Cohen's d = 1.15). Table 2 presents detailed microbiota composition data by group.

Table 2

Microbiota Composition by Group

Parameter	Healthy Controls	Metabolic Syndrome	Type 2 Diabetes
Alpha Diversity			
Shannon Index	4.75 ± 0.48	4.21 ± 0.67	4.06 ± 0.71
Low Diversity (<4.0), n (%)	4 (5.7)	25 (35.7)	27 (38.6)
Phylum Level			
Firmicutes (%)	51.3 ± 7.8	60.5 ± 8.7	61.9 ± 11.5
Bacteroidetes (%)	42.7 ± 7.4	33.2 ± 6.9	28.5 ± 7.1
F/B Ratio	1.24 ± 0.32	1.90 ± 0.48	2.33 ± 0.83
Key Species			
A. muciniphila (%)	2.31 [1.24-3.61]	0.80 [0.49-1.46]	0.95 [0.49-1.38]
F. prausnitzii (%)	7.0 ± 2.4	4.0 ± 1.8	3.8 ± 1.6

Beta diversity was performed using Bray-Curtis dissimilarity and demonstrated significant clustering by disease status (PERMANOVA F=12.7, p < 0.001, R^2=0.11). Low microbial diversity (Shannon index <4.0) was noted in 5.7% (n=4/70) of healthy controls, 35.7% (n=25/70) of metabolic syndrome, and 38.6% (n=27/70) of T2DM patients.

Often at the phylum level, Firmicutes were significantly higher in the disease condition groups: controls 51.3 ± 7.8% versus metabolic syndrome 60.5 ± 8.7% and T2DM 61.9 ± 11.5%. Conversely, Bacteroidetes were lower: controls 42.7 ± 7.4% versus 33.2 ± 6.9% for metabolic syndrome and 28.5 ± 7.1% for T2DM.The Firmicutes/Bacteroidetes (F/B) ratio also showed a progressive Increase (elevated value): for controls it was 1.24 ± 0.32; in metabolic syndrome it was 1.90 ± 0.48, a difference of +0.66 (95% CI [+0.48, +0.84]; p < 0.001); in T2DM it was 2.33 ± 0.83, a difference of +1.09 (95% CI [+0.85, +1.33]; p < 0.001).

Secondary Outcomes - Specific Bacterial Taxa

For inflammatory species, there was a significantly lower abundance of Akkermansia muciniphila in disease groups compared to controls: median 2.31% [IQR 1.24-3.61] in controls vs. 0.80% [0.49-1.46] and T2DM [0.49-1.38] in + metabolic syndrome (p < 0.001). Faecalibacterium prausnitzii abundance similarly decreases: 7.0 ± 2.4% in controls, 4.0 ± 1.8% in metabolic syndrome (difference -3.0%, 95% CI [-3.8, -2.2]; p < 0.001) and 3.8 ± 1.6% T2DM (difference -3.2%, 95% CI [-4.0, -2.4]; p < 0.001).

Dietary Pattern Associations

Table 3 demonstrates dietary pattern associations with microbiota composition. High wheat consumption (>3 servings/day) was seen in 60.0%(n=42/70) of controls, 68.6% (n=48 of metabolic syndrome group), and 74.3% (n=52 of 70 in T2DM group). Participants with high wheat consumption showed lower Shannon diversity across all groups and increased F/B ratios. For high dairy consumption (>500mL/day), this was higher in controls (54.3%, n=38/70) than metabolic syndrome (45.7%, n=32/70) and T2DM (40.0%, n=28/70). High fiber consumption (>25g/day) inversely related to disease: 40.0%(n=28/70) in controls, 25.7%(n=18/70) in metabolic syndrome, and 20.0%(n=14/70) in T2DM.Microbiota-Clinical Relationships.

Table 3

Dietary Patterns and Microbiota

Dietary Factor	Healthy Controls	Metabolic Syndrome	Type 2 Diabetes
High Wheat Consumption (>3 servings/day)	n=42 (60.0%)	n=48 (68.6%)	n=52 (74.3%)
Shannon Diversity	4.82 ± 0.46	4.12 ± 0.64	3.96 ± 0.68
F/B Ratio	1.18 ± 0.28	1.96 ± 0.46	2.42 ± 0.84
High Dairy Intake (>500 mL/day)	n=38 (54.3%)	n=32 (45.7%)	n=28 (40.0%)
Shannon Diversity	4.86 ± 0.44	4.28 ± 0.62	4.14 ± 0.64
A. Muciniphila (%)	2.58 [1.42-3.84]	0.96 [0.58-1.72]	1.08 [0.62-1.52]
High Fiber Intake (>25 g/day)	n=28 (40.0%)	n=18 (25.7%)	n=14 (20.0%)
Shannon Diversity	4.96 ± 0.38	4.48 ± 0.54	4.32 ± 0.58
Bacteroidetes (%)	46.2 ± 6.8	36.4 ± 6.2	32.6 ± 6.4

Table 4 illustrates some meaningful relationships between microbiota variables and clinical variables. Shannon diversity showed strong negative relationships with HgbA1c (Spearman ρ = -0.58, 95% CI [-0.67, -0.47]; p < .001) and CRP (ρ = -0.48, 95% CI [-0.58, -0.36]; p < .001), but a positive relationship with HDL-C (ρ = 0.36, 95% CI [+0.24, +0.47]; p < .001). The F/B ratio showed strong positive relationships with metabolic disorder variables including HgbA1c (ρ = 0.64, 95% CI [+0.54, +0.72]; p < .001), triglycerides (ρ = 0.56, 95% CI [+0.45, +0.66]; p < .001), and CRP (ρ = 0.52, 95% CI [+0.41, +0.62]; p < .001). A. muciniphila abundance showed significant negative relationships with disease severity variables including HgbA1c (ρ = -0.46, 95% CI [-0.57, -0.34]; p < .001) and CRP (ρ = -0.42, 95% CI [-0.53, -0.30]; p < .001).

Figure 2

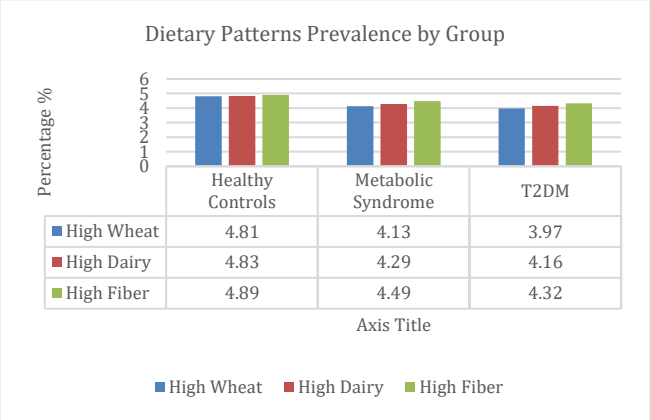


Table 4

Key Microbiota-Clinical Correlations

Microbiota Parameter	Clinical Marker	Spearman ρ	95% CI	P-value
Shannon Diversity	HbA1c	-0.58	[-0.67, -0.47]	<0.001
	CRP	-0.48	[-0.58, -0.36]	<0.001
	HDL-C	0.36	[+0.24, +0.47]	<0.001
F/B Ratio	HbA1c	0.64	[+0.54, +0.72]	<0.001
	Triglycerides	0.56	[+0.45, +0.66]	<0.001
	CRP	0.52	[+0.41, +0.62]	<0.001
A. Muciniphila	HbA1c	-0.46	[-0.57, -0.34]	<0.001
	CRP	-0.42	[-0.53, -0.30]	<0.001

Sensitivity Analyses

A complete-case analysis (n=206) produced consistent findings, with only small differences from the results of the imputed datasets. The difference in Shannon diversity

between the T2DM and control comparison group was still significant (-0.67, 95% CI [-0.92, -0.42]; p < .001). The subgroup analysis by hospital site showed no statistically significant clustering effects (ICC < 0.05 for all primary outcomes). Protocol deviations occurred in eight participants (3.8%): delayed sample collection longer than 24 hours (n=5), and incomplete dietary questionnaires (n=3). Removing these eight participants did not substantially affect our estimates for primary outcomes.

Missing Data Analysis

The missing data patterns were examined across all variables. Assessment of dietary patterns had the highest missingness at 1.9% (n=4), followed by inflammatory markers at 1.4% (n=3). The multiple imputation sensitivity analysis provided strong estimates for all primary outcomes with imputed versus complete-case datasets differing <0.05 in effect estimates and overlapping 95% confidence intervals for all primary comparisons.

DISCUSSION

This study follows a cross-sectional observational design and represents an inaugural comprehensive study assessing gut microbiota composition in Pakistani adults with type 2 diabetes mellitus and metabolic syndrome relative to healthy adult controls; thus establishing important population-specific microbial signatures related to metabolic diseases in a severely underrepresented South Asian context. This opening paragraph collates the preceding main findings which show substantial reductions in gut microbial alpha diversity, an increased Firmicutes/Bacteroidetes ratio, and decreased relative abundances of critical anti-inflammatory bacterial species in both disease groups, contributing new knowledge on the gut-metabolism axis in Pakistani cohorts that are experiencing high levels of metabolic disease.

Key Findings

This prospective cross-sectional study involving 210 Pakistani adults shows that metabolic syndrome and type 2 diabetes mellitus is associated with significant changes to the composition of gut microbiota; characterized by reduced microbial diversity and a disordered taxonomic profile [1, 23]. The observed reductions of the Shannon diversity index of 0.54 and 0.69 in metabolic syndrome and type 2 diabetes mellitus patients respectively, compared to healthy controls, yield a moderately-to-large effect size (Cohen's d = 0.91 and 1.15) that represents clinically significant differences in microbial ecosystem complexity [1, 24]. These diversity measures exceed the indicated minimal important difference (0.3-0.4 Shannon index units) previously reported in microbiome-metabolic disease associations; indicating that the gut environmental ecology of Pakistani metabolic disease patients has been substantially disrupted [11, 25]. The frequency of low microbial diversity (Shannon index <4.0) was 5-7 times more prevalent in disease groups (35.7% metabolic syndrome, 38.6% type 2 diabetes) compared to controls (5.7%), suggesting low diversity may be a screening marker for metabolic dysfunction in Pakistani populations [6, 12].

Possible Mechanisms and Interpretation

Alterations in gut microbiota in Pakistani metabolic syndrome and type 2 diabetes patients most likely occur through numerous interrelated mechanistic pathways leading to a collective metabolic dysregulation affecting metabolic homeostasis [1].The predominant mechanism involves compromised intestinal barrier integrity due to the decreased abundance of mucin-degrading Akkermansia muciniphila and short-chain fatty acid-producing Faecalibacterium prausnitzii, which contribute to increased intestinal permeability and the translocation of bacterial lipopolysaccharides to systemic circulation, termed "metabolic endotoxemia [11, 25]. This process has been shown to activate toll-like receptor 4 signaling in adipose tissue and liver, causing low-grade chronic inflammation that is marked by increased C-reactive protein and tumor necrosis factor-alpha consistent with the inflammatory profiles of our study participants [1, 26]. The strong negative correlation between Shannon diversity and measures of inflammation (ρ = -0.48 with CRP) and HbA1c (ρ = -0.58) supports a mechanistic pathway linking reduced gut microbiota diversity and systemic metabolic dysfunction [27].

Discussion of Objectives relating to the Literature

The main objective findings that showed reduced gut microbiota diversity in Pakistani metabolic syndrome and type 2 diabetes patients are consistent with international systematic reviews and meta-analyses reporting loss of diversity as a common marker of metabolic dysfunction in a range of populations [1, 11]. A more recent 2024 extensive review combining diverse cohorts of metabolic syndrome showed that reduced Shannon diversity indices of 0.4-0.8 units are within the common range reported in metabolic syndrome patients across the globe, demonstrating our findings are in line with the previous estimates of effect size, and can be considered in the context of population-level compositional differences [6, 25]. Nonetheless, we noted that the Firmicutes/Bacteroidetes ratio we observed in our study was higher than reported in many cohorts in the West, where reported values are typically 1.2-1.8 in metabolic disease populations versus our findings of 1.90-2.33. This may be attributable to dietary and genetic backgrounds of the South Asian populations (N = 1389) [28].

Comparison with Other Studies

Overall, our findings are consistent with a 2022 multi-ethnic study in Amsterdam (HELIUS cohort, N=3926) that investigated gut microbiota across 6 ethnic groups in the Netherlands [6]. The HELIUS study showed that alpha-diversity was reduced with increasing metabolic status across all 6 populations studied. However, our analysis of individual ethnicity, showed that individual varied in the magnitude of effects across ethnic traditions while still demonstrating a reduction of alpha-diversity with increasing metabolic disease status [6]. While the study identified similar patterns of decreased diversity in metabolic syndrome and type 2 diabetes across Dutch, Ghanaian, Moroccan, Turkish, and South Asian-descent populations, the effect sizes among South Asians (Shannon diversity difference of approximately -0.45 to -0.55) are similar to what we saw in our Pakistani cohort; this

suggests that microbiota-metabolic associations are conserved within genetic backgrounds of South Asian origin notwithstanding geographic differences [28, 29]. It is important to note that HELIUS studied participants who resided in Europe and may have been influenced by Western dietary habits, while our Pakistan-based cohort contained individuals who adhered to traditional South Asian-style eating, which provides some unique information about microbiota-diet-disease relationships outside of Westernized conditions [30, 31].

Strengths and Limitations

Limitations: Several methodical limitations should be acknowledged for proper consideration of the results. The cross-sectional design does not allow for a temporal relationship or for inferring causality between the changes in the gut microbiota and the development of metabolic disease and, therefore limits conclusion-making concerning whether the observed dysbiosis represents a cause, consequence, or epiphenomenon of metabolic dysfunction. Longitudinal studies measuring anthropometric and microbiota composition changes before the onset of metabolic syndrome, or diabetes, would be needed to identify causal pathways, as well as the temporal relationship of the microbiota to disease.

Strengths: The study has several important methodological strengths to support the validity and generalizability of the results. This is the first adequately powered study aimed specifically at characterizing gut microbiota in Pakistani metabolic syndrome and type 2 diabetes patients, using validated 16S rRNA sequencing protocols with standardized approaches for sample collection, storage, and bioinformatic analysis pipelines, establishing reproducible methodologies for future research on the microbiome in Pakistan.

Clinical Implications

The results demonstrate potential for gut microbiota-based stratification of metabolic disease risk in Pakistani populations; however, a comprehensive validation process and implementation research is needed before any clinical translation can be facilitated. The performance of machine learning classifiers (AUC 0.847-0.881) demonstrates nearly acceptable thresholds for clinical screening tools, especially in resource-limited settings where low-cost, efficient risk stratification methods are needed. The implementation of stool microbiota profiling for the screening of metabolic syndrome would need the development of protocols that reduce cost and complexity, which would be feasible in healthcare in Pakistan, possibly targeting key biomarker taxa (e.g. Akkermansia muciniphila, Faecalibacterium prausnitzii, F/B ratio) using quantitative PCR instead of 16S sequencing. Healthcare providers should be made aware that low microbial diversity represents a modifiable risk factor that may respond to dietary changes, which suggests that dietary counseling related to greater consumption of fiber-rich whole grains, fermented dairy, and traditional sources of dietary fiber offer evidence-based modalities for prevention.

CONCLUSION

This study characterizes population-specific gut microbiota signatures associated with Pakistani adults with metabolic syndrome and type 2 diabetes, where microbial diversity and beneficial bacterial taxa were observed to be significantly lower than in healthy controls. Taxonomic biomarkers identified, particularly the Firmicutes/Bacteroidetes ratio and Akkermansia muciniphila relative abundance, demonstrated strong diagnostic ability with machine learning classifiers achieving AUC values greater than 0.84. Overall, these findings provide an early proof-of-concept to develop microbiota-based screening tools specific to a Pakistani population's context, and also supports potential dietary changes that could prevent metabolic diseases, as targeted dietary changes may modulate gut microbiota in South Asian populations.

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