

INTEGRATED ASSESSMENT OF ACE I/D POLYMORPHISM AND GUT MICROBIAL DIVERSITY IN RELATION TO METABOLIC, INFLAMMATORY, PHYSIOLOGICAL, AND RENAL HISTOPATHOLOGICAL FINDINGS IN TYPE 2 DIABETES MELLITUS: PUBLIC HEALTH IMPLICATIONS FOR RISK CHARACTERIZATION AND EARLY DETECTION IN PAKISTAN

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) involves chronic hyperglycemia with metabolic, inflammatory, vascular, and organ-level abnormalities.

Objective: To assess ACE I/D genotype and allele distributions and gut microbial diversity in adults with T2DM and non-diabetic controls and examine their associations with metabolic, inflammatory, physiological, and renal histopathological findings.

Methods: A comparative cross-sectional study was included 200 adults (150 with T2DM and 50 controls). Demographic and clinical characteristics were assessed in a tertiary care hospital of Pakistan during the period from January 2026 to June 2026. ACE I/D polymorphism was determined by polymerase chain reaction, while stool specimens underwent 16S rRNA-based microbial profiling. Renal tissue specimens were assessed histopathologically. Group comparisons, correlations, and multivariable regression analyses were performed.

Results: Compared with controls, participants with T2DM had higher BMI, glucose, HbA1c, triglycerides, total cholesterol, LDL-C, CRP, IL-6, systolic and diastolic blood pressure, and lower HDL-C. Overall ACE genotype distribution did not differ significantly ($\chi^2=5.72$, $df=2$, $p=0.057$), whereas the D-allele frequency was higher in T2DM (60.7% vs. 46.0%; $p=0.010$; OR=1.81, 95% CI 1.15–2.86). HbA1c, triglycerides, systolic blood pressure, and CRP differed across ACE genotypes. *Faecalibacterium*, *Roseburia*, and *Akkermansia* were reduced, whereas Enterobacteriaceae and *Escherichia/Shigella* were increased in T2DM. Microbial diversity and selected taxa correlated significantly with HbA1c and CRP. Renal inflammatory, vascular, degenerative, edematous, and fibrotic findings were more frequent in T2DM.

Conclusion: ACE I/D variation, gut microbial alterations, metabolic and inflammatory characteristics, physiological parameters, and renal histopathological findings showed significant associations in T2DM, supporting integrated risk characterization and further investigation.

KEYWORDS: Type 2 diabetes mellitus; ACE I/D polymorphism; gut microbiota; microbial diversity; inflammation; HbA1c.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, and even both. Diagnostic assessment is based on established glycemic criteria, including glycated hemoglobin (HbA1c), fasting plasma glucose, oral glucose tolerance testing, and, in appropriate clinical circumstances, random plasma glucose (1). The metabolic abnormalities associated with T2DM extend beyond hyperglycemia and include dyslipidemia, hypertension, systemic inflammation, endothelial dysfunction, and progressive organ involvement. Chronic inflammatory activity has been investigated extensively in T2DM, with circulating markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) associated

with metabolic abnormalities and disease-related complications (2,3). The renin–angiotensin system is an important regulator of vascular tone, blood pressure, fluid balance, and renal physiology. Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II and therefore represents a central component of the renin–angiotensin system. The ACE insertion/deletion (I/D) polymorphism is in intron 16 and produces three genotypes: II, ID, and DD. The D allele has been associated with higher circulating ACE concentrations, although associations between ACE I/D genotype and diabetes-related phenotypes have differed among populations (4-6).

Interpretation of ACE I/D genotype studies also requires attention to laboratory methodology. Conventional PCR using flanking primers can preferentially amplify the shorter D allele, resulting in potential misclassification of some ID individuals as DD. Previous methodological studies have therefore recommended confirmation of DD results using insertion-specific approaches (7,8). The gut microbiota has also emerged as an important component of metabolic research. Observational studies have identified differences in microbial diversity and taxonomic composition between individuals with T2DM and non-diabetic individuals. *Faecalibacterium*, *Roseburia*, and *Akkermansia* have frequently been reported in association with metabolic health, while *Escherichia/Shigella* and other taxa have been reported in association with T2DM. However, substantial heterogeneity exists between populations and studies, and microbiome findings remain influenced by dietary, pharmacological, environmental, and methodological factors (10-12).

Renal involvement is an important component of diabetes-related morbidity. Histopathological abnormalities in individuals with T2DM can involve glomerular, vascular, tubular, and interstitial compartments. Renal biopsy studies have demonstrated that patients with T2DM may have diabetic nephropathy, non-diabetic renal disease, or combinations of pathological processes (13-15). A study conducted in Karachi demonstrated heterogeneous renal pathology among patients with T2DM who underwent clinically indicated renal biopsy, including diabetic glomerulosclerosis, vascular changes, tubular injury, and interstitial inflammation (15). The renal renin–angiotensin system is particularly relevant to diabetes-related renal injury because ACE is expressed within renal tissue and contributes to local angiotensin II generation. Previous human renal tissue studies have investigated ACE expression and localization in diabetic nephropathy (16). Despite these separate lines of evidence, limited research has evaluated ACE I/D polymorphism, gut microbial diversity, metabolic and inflammatory characteristics, physiological parameters, and renal histopathological findings together within the same study population. The present study therefore assessed these domains among adults with T2DM and non-diabetic controls in Pakistan.

METHODOLOGY

A comparative cross-sectional study was conducted in a tertiary care hospital of Pakistan during the period from January 2026 to June 2026 among 200 adults comprising 150 participants with T2DM and 50 non-diabetic controls. T2DM was defined based on documented clinical diagnosis and/or accepted glycemic diagnostic criteria (1). Written informed consent was obtained from participants. Participant information and biological specimens were coded to maintain confidentiality. Participants with type 1 diabetes mellitus, gestational diabetes, acute infection or inflammatory illness, major gastrointestinal disease or gastrointestinal surgery, malignancy, severe systemic disease, or recent exposure to medications likely to substantially alter gut microbial composition were excluded. The control group comprised individuals without diabetes according to the study eligibility criteria. Demographic and clinical information included age, sex, BMI, diabetes duration, family history of diabetes, antihyperglycemic medication use, and blood pressure. BMI was calculated from measured body weight and height and expressed in kg/m². Blood pressure was measured after rest and recorded as systolic blood pressure (SBP) and diastolic blood pressure (DBP). Fasting blood samples were analyzed for fasting glucose, HbA1c, triglycerides (TG), total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), CRP, and IL-6. The biochemical and inflammatory measurements were used to characterize the metabolic and inflammatory profiles of the T2DM and control groups and to evaluate their relationships with ACE genotype and microbial measures. Genomic DNA was analyzed for the ACE I/D polymorphism using PCR.

The primer sequences were:

Forward primer:

5'-CTGGAGACCACTCCCATCCTTTCT-3'

Reverse primer:

5'-GATGTGGCCATCACATTCGTCAGAT-3'

Each 25-μL PCR reaction contained:

- 10 pmol of each primer;
- 1.5 mM MgCl₂;
- 0.2 mM of each dNTP;
- 1 U hot-start Taq DNA polymerase;
- approximately 2 μL template DNA; and
- nuclease-free water to the final volume.

The thermal cycling protocol consisted of:

- initial denaturation at 95°C for 2 minutes;
- 30 cycles of denaturation at 94°C for 30 seconds;
- annealing at 58°C for 30 seconds;
- extension at 72°C for 45 seconds; and
- final extension at 72°C for 9 minutes.

The expected amplification products were approximately 490 bp for the I allele and 190 bp for the D allele. Genotypes were classified as II, ID, or DD according to the amplification pattern. The potential for preferential D-allele amplification in conventional ACE I/D PCR was considered in interpretation because this limitation has been documented previously (7,8). Stool specimens were collected in sterile containers and processed for microbial DNA analysis. Bacterial community profiling was performed using 16S rRNA gene-based sequencing. Microbial diversity was assessed using the Shannon diversity index, Simpson diversity index, and observed taxa. Relative abundance was assessed for the selected taxa:

- *Faecalibacterium*;
- *Roseburia*;
- *Akkermansia*;
- Enterobacteriaceae; and
- *Escherichia/Shigella*.

Microbial measures were subsequently evaluated in relation to glycemic and inflammatory variables. Renal tissue specimens included in the study were examined histopathologically. The recorded renal histopathological findings comprised:

- inflammatory-cell infiltration;
- vascular congestion;
- endothelial/vascular changes;
- tissue edema;
- focal tissue degeneration; and
- fibrotic changes.

The findings were recorded according to their presence or absence in the evaluated tissue. A histopathological severity score was calculated from the recorded abnormalities, with higher scores representing a greater number of documented histopathological abnormalities. The renal findings were analyzed as actual tissue findings observed in the study population. They were not classified solely based on the present analysis as diabetic nephropathy, because individual inflammatory, vascular, degenerative, edematous, and fibrotic findings may occur across different renal pathological processes (13-15). Continuous variables were summarized as mean $\pm$ standard deviation, while categorical variables were presented as frequencies and percentages. Between-group comparisons were performed using appropriate statistical tests for continuous and categorical variables. Chi-square or Fisher's exact tests were used for categorical comparisons where appropriate. ACE genotype and allele frequencies were calculated for both study groups. Genotype distribution was assessed using chi-square testing. Hardy–Weinberg equilibrium was assessed in the control group. Within the T2DM group, differences in HbA1c, TG, SBP, CRP, and renal histopathological findings across ACE genotype groups were assessed. Correlations between microbial measures and HbA1c or CRP were evaluated. Regression coefficients were reported with 95% confidence intervals and p-values. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

The study comprised 150 participants with T2DM and 50 non-diabetic controls. Mean age did not differ significantly between the groups. BMI and the proportion reporting a family history of diabetes were significantly higher among participants with T2DM. The mean duration of diabetes among participants with T2DM was 7.2 ± 4.8 years, and 129 (86.0%) were receiving antihyperglycemic medication as shown in table 1.

Table 1. Demographic and clinical characteristics of study participants

Variable	T2DM (n=150)	Controls (n=50)	p-value
Age, years	52.4 ± 9.1	49.8 ± 8.7	0.081
Male, n (%)	82 (54.7)	27 (54.0)	0.934
BMI, kg/m ²	28.7 ± 4.3	25.9 ± 3.7	<0.001
Diabetes duration, years	7.2 ± 4.8	—	—
Family history, n (%)	91 (60.7)	17 (34.0)	0.001
Antihyperglycemic medication, n (%)	129 (86.0)	—	—

Participants with T2DM had significantly higher fasting glucose and HbA1c than controls. TG, TC, LDL-C, CRP, and IL-6 were also significantly higher, whereas HDL-C was significantly lower than shown in table 2.

Table 2. Biochemical and inflammatory characteristics

Variable	T2DM (n=150)	Controls (n=50)	p-value
Fasting glucose, mg/dL	164.8 ± 42.6	96.7 ± 10.8	<0.001
HbA1c, %	8.1 ± 1.7	5.4 ± 0.4	<0.001
TG, mg/dL	186.3 ± 72.5	128.5 ± 39.7	<0.001
TC, mg/dL	205.7 ± 41.2	183.6 ± 32.5	0.001
LDL-C, mg/dL	124.5 ± 34.8	109.7 ± 27.4	0.007
HDL-C, mg/dL	39.8 ± 8.9	46.7 ± 7.8	<0.001
CRP, mg/L	4.7 ± 2.9	2.1 ± 1.4	<0.001
IL-6, pg/mL	5.9 ± 3.2	3.2 ± 1.5	<0.001

SBP and DBP were significantly higher among participants with T2DM. Hypertension was also significantly more frequent in the T2DM group as shown in table 3.

Table 3. Physiological characteristics

Variable	T2DM (n=150)	Controls (n=50)	p-value
SBP, mmHg	139.4 ± 17.2	121.8 ± 12.6	<0.001
DBP, mmHg	86.7 ± 10.5	77.4 ± 8.6	<0.001
Hypertension, n (%)	79 (52.7)	12 (24.0)	<0.001

The observed ACE genotype frequencies among participants with T2DM were 20.7% for II, 37.3% for ID, and 42.0% for DD. Among controls, the corresponding frequencies were 32.0%, 44.0%, and 24.0%. The overall genotype distribution did not reach statistical significance between groups ($\chi^2=5.72$, df=2, p=0.057) as shown in table 4.

Table 4. ACE I/D genotype distribution

ACE genotype	T2DM (n=150)	Controls (n=50)
II	31 (20.7%)	16 (32.0%)
ID	56 (37.3%)	22 (44.0%)
DD	63 (42.0%)	12 (24.0%)
Overall χ^2	5.72	
df	2	
p-value	0.057	

The D allele was significantly more frequent among participants with T2DM than controls (60.7% vs. 46.0%; p=0.010; OR=1.81, 95% CI 1.15–2.86) as shown in table 5.

Table 5. ACE I/D allele distribution

Allele	T2DM	Controls	p-value	OR (95% CI)
I	39.3%	54.0%	0.010	Reference
D	60.7%	46.0%	0.010	1.81 (1.15–2.86)

The control genotype distribution was compatible with Hardy–Weinberg equilibrium ($\chi^2\approx 0.65$, p=0.419).

Within the T2DM group, HbA1c, TG, SBP, and CRP differed significantly across ACE genotype groups, with the highest reported values observed among DD participants as shown in table 6.

Table 6. Metabolic, inflammatory, and physiological characteristics according to ACE genotype

Variable	II (n=31)	ID (n=56)	DD (n=63)	p-value
HbA1c, %	7.5 ± 1.3	7.9 ± 1.5	8.5 ± 1.8	0.006
TG, mg/dL	169.2 ± 58.4	181.6 ± 64.7	198.8 ± 79.3	0.041
SBP, mmHg	133.1 ± 14.6	138.2 ± 16.2	143.5 ± 18.3	0.012
CRP, mg/L	3.9 ± 2.3	4.4 ± 2.6	5.2 ± 3.2	0.048

T2DM participants demonstrated significantly lower microbial diversity across all three reported alpha-diversity measures as shown in table 7.

Table 7. Gut microbial diversity

Diversity measure	T2DM	Controls	p-value
Shannon index	3.41 ± 0.62	3.87 ± 0.55	<0.001
Simpson index	0.78 ± 0.08	0.83 ± 0.06	<0.001
Observed taxa	118 ± 24	137 ± 21	<0.001

Selected bacterial taxa also differed between the groups as shown in table 8.

Table 8. Selected gut microbial taxa

Taxon	T2DM	Controls	Adjusted p-value
<i>Faecalibacterium</i>	2.8	5.1	0.008
<i>Roseburia</i>	1.9	3.6	0.011
<i>Akkermansia</i>	1.1	2.4	0.019
Enterobacteriaceae	4.7	2.1	0.014
<i>Escherichia/Shigella</i>	3.2	1.4	0.021

Shannon diversity showed inverse correlations with HbA1c and CRP. *Faecalibacterium* and *Roseburia* were also inversely correlated with HbA1c and CRP, whereas Enterobacteriaceae and *Escherichia/Shigella* demonstrated positive correlations as shown in table 9.

Table 9. Correlations between microbial measures and HbA1c and CRP

Microbial measure	HbA1c, r	CRP, r	p-value
Shannon diversity	-0.31	-0.27	<0.001
<i>Faecalibacterium</i>	-0.28	-0.24	0.002
<i>Roseburia</i>	-0.22	-0.20	0.008
Enterobacteriaceae	0.25	0.29	0.001
<i>Escherichia/Shigella</i>	0.21	0.26	0.004

Renal histopathological examination demonstrated significantly higher frequencies of several tissue abnormalities among participants with T2DM compared with controls. Inflammatory-cell infiltration was the most frequently recorded finding in the T2DM group, followed by vascular congestion and endothelial/vascular changes as shown in table 10.

Table 10. Renal histopathological findings

Histopathological finding	T2DM (n=150)	Controls (n=50)	p-value
Inflammatory-cell infiltration	68 (45.3%)	8 (16.0%)	<0.001
Vascular congestion	61 (40.7%)	7 (14.0%)	0.001
Endothelial/vascular changes	57 (38.0%)	6 (12.0%)	0.001
Tissue edema	49 (32.7%)	5 (10.0%)	0.002
Focal tissue degeneration	43 (28.7%)	4 (8.0%)	0.003
Fibrotic changes	31 (20.7%)	3 (6.0%)	0.018

Several renal histopathological findings differed significantly across ACE genotype groups. Inflammatory-cell infiltration increased from 29.0% among II participants to 57.1% among DD participants. Similar genotype-related patterns were observed for vascular congestion, endothelial/vascular changes, and focal tissue degeneration as shown in table 11.

Table 11. Renal histopathological findings according to ACE genotype

Histopathological finding	II (n=31)	ID (n=56)	DD (n=63)	p-value
Inflammatory-cell infiltration	9 (29.0%)	23 (41.1%)	36 (57.1%)	0.028
Vascular congestion	8 (25.8%)	21 (37.5%)	32 (50.8%)	0.049
Endothelial/vascular changes	7 (22.6%)	19 (33.9%)	31 (49.2%)	0.038

Tissue edema	6 (19.4%)	17 (30.4%)	26 (41.3%)	0.083
Focal tissue degeneration	5 (16.1%)	14 (25.0%)	24 (38.1%)	0.049
Fibrotic changes	3 (9.7%)	10 (17.9%)	18 (28.6%)	0.071

The renal histopathological severity score differed significantly among ACE genotype groups as shown in table 12.

Table 12. Renal histopathological severity according to ACE genotype

ACE genotype	Severity score, mean $\pm$ SD	p-value
II	1.42 $\pm$ 0.71	
ID	1.83 $\pm$ 0.82	
DD	2.31 $\pm$ 0.91	0.001

Multivariable regression demonstrated significant associations between ACE DD genotype and HbA1c and SBP. Lower microbial diversity was associated with higher HbA1c, while Enterobacteriaceae and *Faecalibacterium* were significantly associated with CRP after adjustment for the specified covariates as shown in table 13.

Table 13. Multivariable regression analysis

Outcome	Main predictor	Adjusted β	95% CI	p-value
HbA1c (%)	ACE DD genotype	0.42	0.15–0.69	0.002
HbA1c (%)	Reduced microbial diversity	0.51	0.22–0.80	<0.001
CRP (mg/L)	Enterobacteriaceae	0.37	0.12–0.62	0.004
CRP (mg/L)	<i>Faecalibacterium</i>	–0.29	–0.51 to –0.07	0.010
SBP (mmHg)	ACE DD genotype	5.1	1.4–8.8	0.007

DISCUSSION

This study evaluated ACE I/D polymorphism and gut microbial diversity in relation to metabolic, inflammatory, physiological, and renal histopathological findings among adults with T2DM and non-diabetic controls. The findings demonstrate significant differences across multiple biological domains and provide evidence for associations between ACE genotype, microbial characteristics, metabolic control, inflammation, blood pressure, and renal tissue abnormalities. Participants with T2DM had substantially higher fasting glucose and HbA1c than controls, confirming the expected difference in glycemic status between the study groups. The T2DM group also demonstrated higher TG, TC, LDL-C, CRP, and IL-6 and lower HDL-C. These findings indicate concurrent metabolic dysregulation and inflammatory activation in the studied population. The physiological findings demonstrated significantly higher SBP and DBP among participants with T2DM, with hypertension present in 52.7% compared with 24.0% among controls. The relationship between diabetes, hypertension, vascular dysfunction, and renal pathology is clinically important because these factors may coexist and contribute to progression of diabetes-related complications. The ACE genotype analysis requires precise interpretation. Although DD genotype was more frequent in the T2DM group, the overall genotype distribution did not reach statistical significance ($p=0.057$). Therefore, the study does not demonstrate a statistically significant difference in overall ACE genotype distribution between T2DM and controls. In contrast, the D allele was significantly more frequent in T2DM, with an OR of 1.81.

This distinction between genotype and allele analyses is important. The significant allele-level finding indicates a difference in D-allele frequency within this study population but should not be described as definitive evidence that ACE genotype independently determines T2DM status. Within participants with T2DM, HbA1c, TG, SBP, and CRP differed significantly across ACE genotype groups. The DD group had the highest observed values for all four variables. Multivariable analysis further demonstrated an association between DD genotype and HbA1c and between DD genotype and SBP after adjustment for age, sex, BMI, diabetes duration, and relevant medication use. The biological relevance of ACE I/D variation is supported by previous evidence demonstrating an association between the ACE I/D polymorphism and circulating ACE levels (4). Nevertheless, the present study did not directly measure ACE activity or angiotensin II concentrations. Therefore, the observed statistical relationships cannot be attributed directly to increased ACE enzymatic activity. The microbial findings demonstrated significantly lower Shannon diversity, Simpson diversity, and observed taxa among participants with T2DM. These findings are consistent with systematic-review evidence demonstrating altered microbial diversity in T2DM. The lower relative abundance of *Faecalibacterium*, *Roseburia*, and *Akkermansia* observed in the T2DM group is also consistent with taxa frequently reported as reduced in T2DM populations. A systematic review of observational studies identified lower abundance of *Faecalibacterium*, *Roseburia*, and *Akkermansia* among taxa reported in T2DM, although substantial heterogeneity exists between studies (12).

The higher abundance of Enterobacteriaceae and *Escherichia/Shigella* in the T2DM group corresponds to patterns reported in previous microbiome research (9,12). However, microbial composition can be influenced by diet, medication, geography, age, and other factors. Consequently, these findings should be interpreted within the context of the studied population rather than generalized to all individuals with T2DM. The correlation analysis demonstrated

that lower Shannon diversity was associated with higher HbA1c and CRP. *Faecalibacterium* and *Roseburia* showed inverse correlations with HbA1c and CRP, whereas Enterobacteriaceae and *Escherichia/Shigella* demonstrated positive correlations. These findings indicate relationships between the measured microbial characteristics and glycemic and inflammatory variables but do not establish directionality. The multivariable findings further demonstrated an association between microbial diversity and HbA1c after adjustment for age, sex, BMI, diabetes duration, and antihyperglycemic medication use (17). Enterobacteriaceae was positively associated with CRP, whereas *Faecalibacterium* was inversely associated with CRP after adjustment.

The renal histopathological findings provide an additional dimension to the study. Inflammatory-cell infiltration, vascular congestion, endothelial/vascular changes, tissue edema, focal tissue degeneration, and fibrotic changes were significantly more frequent among participants with T2DM than controls. Renal pathology associated with T2DM can involve multiple compartments, including glomerular, vascular, tubular, and interstitial structures (18). Renal biopsy studies have also demonstrated that T2DM can be associated with heterogeneous renal lesions, including diabetic glomerulosclerosis, vascular abnormalities, tubular injury, interstitial inflammation, and non-diabetic renal disease (13,19). The present findings are therefore interpreted as documented renal histopathological abnormalities observed in the study population. The presence of inflammatory, vascular, degenerative, edematous, or fibrotic changes should not individually be equated with a complete diagnosis or pathological classification of diabetic nephropathy unless that diagnosis was specifically established through the full pathological assessment.

The observed association between ACE DD genotype and renal histopathological severity is biologically plausible because ACE contributes to local renal renin–angiotensin system activity. Previous renal tissue studies have investigated ACE expression in diabetic nephropathy (20). However, the present study assessed ACE genotype rather than tissue ACE expression or enzymatic activity, and therefore the underlying biological mechanism cannot be established from these data. Further methodological consideration concerns conventional ACE I/D PCR. Previous studies have demonstrated that conventional PCR can misclassify some ID samples as DD because of preferential amplification of the shorter D allele (7,8). Hence, the integrated results demonstrate that T2DM in this population was associated with metabolic abnormalities, inflammatory changes, altered blood pressure, reduced gut microbial diversity, differential abundance of selected bacterial taxa, and increased frequencies of renal histopathological abnormalities. The ACE DD genotype was additionally associated with several metabolic, physiological, and renal histopathological characteristics within the T2DM group.

The findings have implications for future diabetes risk characterization and research in Pakistan.

The high burden of metabolic abnormalities observed among participants with T2DM emphasizes the importance of early identification of hyperglycemia, dyslipidemia, hypertension, and inflammatory risk factors within diabetes prevention and management programs. The higher D-allele frequency and genotype-specific associations observed in this study suggest that ACE I/D polymorphism warrants further investigation as a potential genetic component of diabetes-related risk characterization. However, the absence of a statistically significant difference in overall genotype distribution means that ACE I/D genotype should not currently be presented as an established diagnostic marker for T2DM. The microbial findings similarly indicate that gut microbial diversity and selected taxa are associated with glycemic and inflammatory characteristics. Systematic reviews have documented associations between T2DM and altered microbial diversity, but substantial heterogeneity across populations and methodologies remain (21). From a public health perspective, the most appropriate application of these findings is therefore risk characterization, rather than immediate implementation of ACE or microbiome testing as population-level screening tools. Future Pakistani studies should evaluate whether genetic and microbial measures provide incremental predictive information beyond established risk factors such as age, BMI, family history, glycemic measures, blood pressure, and lipid profile. The renal findings further emphasize the importance of integrating renal assessment into diabetes care and research. Where renal tissue is clinically indicated, histopathological characterization can provide information about the underlying pattern of renal injury and distinguish between diabetic, non-diabetic, and mixed renal disease (13-15).

This study has several strengths, including the inclusion of both T2DM participants and non-diabetic controls and the integrated assessment of ACE I/D genotype and allele distributions, metabolic and inflammatory biomarkers, physiological parameters, gut microbial diversity and selected taxa, and renal histopathological findings. The study further examined microbial associations with HbA1c and CRP, evaluated renal histopathological findings across ACE genotypes, and used multivariable regression models incorporating prespecified clinical covariates, thereby enabling assessment of relationships across genetic, microbial, metabolic, inflammatory, physiological, and tissue-level domains within the same population. However, several limitations should be considered. The cross-sectional design precludes determination of temporal relationships or causality. The sample size of 200 participants (150 with T2DM and 50 controls), particularly the smaller control group, may limit statistical precision for some comparisons. Conventional ACE I/D PCR may preferentially amplify the D allele and potentially misclassify ID individuals as DD if insertion-specific confirmation was not performed (7,8). Although 16S rRNA-based profiling provides taxonomic information, it does not establish microbial functional activity, and microbial composition may be influenced by diet, medications, age, geographic factors, and other unmeasured variables; therefore, residual confounding cannot be

excluded. The renal histopathological findings represent actual tissue abnormalities recorded in the study but do not constitute a complete standardized classification of diabetic nephropathy, as renal pathology in T2DM may include diabetic, non-diabetic, or mixed lesions (13-15). Finally, replication in larger and preferably prospective Pakistani cohorts is needed before ACE genotype or microbiome characteristics are considered for clinical risk prediction or population-level screening.

CONCLUSION

This study provides an integrated assessment of ACE I/D polymorphism, gut microbial diversity, metabolic characteristics, inflammatory biomarkers, physiological parameters, and renal histopathological findings among adults with T2DM and non-diabetic controls. Participants with T2DM demonstrated significantly greater metabolic and inflammatory abnormalities, higher blood pressure, reduced gut microbial diversity, differential abundance of selected bacterial taxa, and higher frequencies of several renal histopathological findings. Although the overall ACE genotype distribution did not differ significantly between groups, the D allele was significantly more frequent among participants with T2DM. Within the T2DM group, DD genotype was associated with higher HbA1c, TG, SBP, and CRP and with several renal histopathological findings.

The microbial findings demonstrated significant relationships between reduced diversity, selected bacterial taxa, HbA1c, and CRP. Multivariable analyses further demonstrate independent statistical associations between ACE DD genotype and HbA1c and SBP and between selected microbial measures and HbA1c or CRP. Taken together, these findings support further investigation of integrated genetic, microbial, metabolic, inflammatory, physiological, and renal tissue markers for risk characterization of T2DM and its complications in Pakistan. However, the cross-sectional design limits causal interpretation, and prospective validation is required before ACE I/D genotype or gut microbial markers can be incorporated into routine clinical or population-level screening.

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