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Urinary Glucose as a Potential Mediator of Infection Risk in Type 2 Diabetes Treated with SGLT2 Inhibitors: Evidence from a Prospective Cohort Study of Immune-metabolic Crosstalk

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ABSTRACT

This study evaluates the association between 24-hour urinary glucose excretion (UGE) and urogenital tract infection (UGTI) risk in patients with type 2 diabetes mellitus receiving sodium-glucose cotransporter 2 inhibitor-containing therapy and explores potential immune-metabolic mechanisms.

This prospective single-center cohort enrolled adults initiating SGLT2 inhibitor-containing therapy between February 2024 and June 2025. Of 412 enrolled patients, 380 were included in the final analysis and followed for up to 12 months. Patients were categorized into quartiles according to 24-hour UGE measured 3 months after treatment initiation. Urinary immune-cell subsets, soluble immune factors, antimicrobial peptides, and glucose-related metabolites were assessed. Time-to-event, restricted cubic spline, mediation, and sensitivity analyses were performed.

Each UGE quartile included 95 patients. The cumulative incidence of pathogen-confirmed UGTI was 10.26% and increased progressively from Q1 to Q4 (3.16%, 7.37%, 12.63%, and 17.89%, respectively). Higher UGE remained independently associated with increased UGTI risk after multivariable adjustment, with a nonlinear positive exposure-response relationship. UGE statistically mediated part of the association between glycemic improvement and infection risk. Higher UGE was also associated with a lower urinary macrophage M1/M2 ratio, reduced interleukin-8 and β-defensin 2 levels, and lower pyruvate, lactate, and citrate levels.

Higher UGE was independently associated with greater UGTI risk and unfavorable urinary immune-metabolic profiles. These observational findings support UGE as a potential clinical mediator but do not establish causality.

Keywords: Immune-metabolic crosstalk; Infection risk; SGLT2 inhibitor; Type 2 diabetes mellitus; Urinary glucose; Urogenital tract infection

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) remains a major global public health challenge. According to the 11th

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edition of the International Diabetes Federation Diabetes Atlas, an estimated 589 million adults aged 20–79 years were living with diabetes worldwide in 2024, and this number is projected to increase substantially by 2050, with China accounting for approximately 141 million cases, ranking first globally.[1] Infection is the second leading cause of death in T2DM patients, behind only cardiovascular and cerebrovascular complications, contributing to 15% to 20% of all-cause mortality.[2] Urogenital tract infection (UGTI) is the most prevalent type of infection in this population: diabetic patients have a 2- to 3.5-fold higher risk of UGTI than non-diabetic individuals, and are more prone to progress to severe infections, such as pyelonephritis and urosepsis, which significantly increase medical burden and mortality risk.[3]

The traditional theory holds that systemic hyperglycemia toxicity is the core mechanism underlying the increased infection susceptibility in diabetic patients. Long-term hyperglycemia inhibits the phagocytic and bactericidal functions of neutrophils and macrophages, disrupts the mucosal barrier and adaptive immune homeostasis through the activation of the polyol pathway, accumulation of advanced glycation end products, and enhanced oxidative stress,[4] resulting in the so-called "diabetes-related immune paralysis."[5] Based on this theory, the academic community generally believes that effective glucose lowering can reverse immune dysfunction and reduce the risk of infection in T2DM patients.

However, the clinical application of sodium-glucose cotransporter 2 inhibitors (SGLT2i) has posed a severe challenge to this traditional theory, forming a long-standing unresolved clinical paradox in the field of endocrinology.[3] SGLT2 inhibitors achieve insulin-independent glucose lowering by inhibiting glucose reabsorption in the renal proximal tubule and increasing urinary glucose excretion (UGE), and have become a cornerstone agent for T2DM management.[6] Multiple large-scale cardiovascular outcome trials (CVOTs) have confirmed that SGLT2i has significant cardiovascular and renal protective benefits. It is listed as the first-line preferred agent for T2DM patients with concurrent cardiovascular and renal diseases by domestic and international guidelines, and its indications have been extended to non-diabetic patients with heart failure and chronic kidney disease,[7] with more than 100 million users worldwide.

At the same time, however, all evidence-based medical data consistently confirm that SGLT2i treatment more than doubles the risk of UGTI in T2DM patients, which is the leading cause of permanent drug discontinuation.[8] Relevant studies have shown that the risk of UGTI in T2DM patients treated with SGLT2i is 2 times higher than that in the placebo group, with a more significant increase in female patients.[9] In addition, SGLT2i is associated with an increased risk of fatal infections, such as Fournier's gangrene and severe pyelonephritis, for which the US Food and Drug Administration (FDA) has issued a black box warning.[10] On the other hand, patients who discontinue SGLT2i treatment have a significantly higher risk of cardio-renal events and all-cause mortality, making infection-related risk a core bottleneck limiting patients' access to target organ protection benefits.

Up to now, research on the mechanism of SGLT2i-related infection has remained at a superficial stage. The traditional hypothesis that "urinary glucose provides a nutrient substrate for pathogens" fails to explain the core contradictory phenomena in clinical practice: first, there is a significant dissociation between UGE and the occurrence of infection, with some patients with high UGE having no infection while some patients with low UGE experiencing repeated severe infections; second, the infection risk persists even after patients achieve glycemic control targets; third, there is no significant difference in UGE between male and female patients, but there is a 2- to 3-fold gender difference in infection risk. More critically, the traditional hypothesis primarily focuses on the pathogen level, often overlooking the host immune defense function, which may have hindered progress in related mechanistic research.

In recent years, the rapid development of the discipline of immune-metabolic crosstalk has provided a brand-new perspective to solve this problem. The core of this theory is that the activation, phagocytosis, sterilization, and other functions of immune cells are highly dependent on intracellular metabolic reprogramming, and the concentration of nutrients in the local microenvironment can directly regulate the metabolic pathways and functional phenotypes of immune cells.[11] Previous studies have confirmed that systemic hyperglycemia can inhibit the antibacterial defense function of immune cells by interfering with their glycolysis reprogramming, which is the core mechanism of diabetic immune paralysis.[12] But for patients treated with SGLT2i, the core metabolic feature

is that while systemic blood glucose is reduced, a persistent high urinary glucose microenvironment appears locally in the urogenital tract, with urinary glucose concentration reaching 100–500 mmol/L,[13] which is much higher than the physiological level and circulating blood glucose concentration.[8] At present, there is no systematic study on whether this local high urinary glucose environment disrupts the host mucosal immune defense through the immune-metabolic crosstalk mechanism, thereby mediating the occurrence of infection.

Based on this, this study proposes the core scientific hypothesis: persistent elevation of urinary glucose induced by SGLT2i treatment is the core biochemical mediator of increased infection susceptibility in T2DM patients. Its mechanism lies not only in providing nutrient substrates for pathogens, but more critically, urinary glucose impairs mucosal immune defense function by remodeling the local immune-metabolic homeostasis of the urogenital tract, inhibiting the activation of the bactericidal phenotype of innate immune cells and reducing the secretion of antimicrobial peptides, which ultimately leads to an increased risk of infection. Through a prospective cohort design, this study investigated the association between UGE and infection risk, explored the potential mediating role of UGE, and examined the findings from the perspective of immune-metabolic crosstalk, with the aim of providing clinical evidence for the safer use of SGLT2 inhibitor-containing therapy.

MATERIALS AND METHODS

Study Subjects

This prospective single-center cohort study was conducted in adults with T2DM who newly initiated SGLT2 inhibitor-containing therapy at our hospital between February 2024 and June 2025. Enrollment was consecutive. The analytic cohort consisted of 380 patients who completed exposure classification and subsequent follow-up. The overall study workflow therefore comprised a recruitment phase, a 3-month exposure ascertainment window for UGE measurement, and a 12-month outcome observation period. The SGLT2 inhibitor-containing regimens used in this cohort mainly included dapagliflozin, canagliflozin, and fixed-dose dapagliflozin/metformin according to routine prescribing practice at our center.

Inclusion criteria were age 18–75 years; T2DM diagnosed according to accepted clinical criteria; initiation of dapagliflozin, canagliflozin, or fixed-dose dapagliflozin/metformin as part of standard glucose-lowering treatment; and ability to complete scheduled visits and specimen collection. Major exclusion criteria were type 1 diabetes or other specific diabetes types, active severe infection at baseline, recurrent urogenital infection within the prior 12 months, severe immunodeficiency, pregnancy or lactation, malignant tumor under active treatment, end-stage renal disease, or other conditions judged by investigators to substantially interfere with follow-up or endpoint adjudication.

To ensure reliable exposure classification, patients who discontinued SGLT2 inhibitor-containing therapy within the initial 3 months for reasons unrelated to infection, had major protocol deviations, or had missing key baseline or 3-month exposure data were excluded from the final analysis. Participants who later discontinued treatment because of infection after exposure classification were retained in the time-to-event analysis, and such discontinuation was analyzed as a secondary clinical outcome.

Patient flow was documented according to STROBE principles. A total of 412 consecutive patients were screened for eligibility. After application of the inclusion and exclusion criteria and completion of the 3-month exposure ascertainment visit, 380 patients were included in the final analytic cohort. Patients excluded before the analytic cohort was established mainly included those with early discontinuation of SGLT2 inhibitor-containing therapy for reasons unrelated to infection, incomplete 24-hour urine collection or missing key exposure data, loss to follow-up before the 3-month visit, withdrawal of consent, or major protocol deviations. Patients who discontinued SGLT2 inhibitor-containing therapy after exposure classification because of infection were retained in the analysis and were also analyzed as a secondary clinical outcome. This landmark-based design was used to ensure that UGE was measured before subsequent endpoint assessment, although potential selection bias related to the 3-month exposure ascertainment window could not be completely excluded.

Follow-up Protocol and Endpoint Definition

All patients underwent a baseline assessment before starting SGLT2 inhibitor-containing therapy, including demographic information, medical history,

medication use, laboratory measurements, and biospecimen collection. Follow-up visits were scheduled at months 1, 3, 6, 9, and 12. The primary endpoint was the first pathogen-confirmed UGTI during follow-up after exposure classification. UGTI included symptomatic bacterial urinary tract infection and clinically compatible genital infection with microbiological support. Symptomatic urinary tract infection required urinary symptoms, such as dysuria, urinary frequency, urgency, suprapubic discomfort, fever, or flank pain, together with a positive urine culture according to routine clinical laboratory criteria. Genital infection required compatible local symptoms or signs and microbiological evidence from genital samples, when available. Asymptomatic bacteriuria alone was not counted as a primary endpoint. Samples considered contaminated by the clinical laboratory, such as those with mixed flora without a predominant pathogen, were repeated whenever clinically feasible and were not adjudicated as confirmed events without supportive clinical evidence. Genital fungal infection and bacterial urinary tract infection were recorded separately for descriptive and subgroup analyses, while the primary endpoint used the first confirmed UGTI as a composite outcome.

An independent endpoint adjudication committee composed of senior physicians from endocrinology, nephrology, and infectious diseases reviewed suspected events using predefined clinical criteria. For patients with multiple infectious episodes, only the first pathogen-confirmed UGTI was counted in the primary time-to-event analysis; subsequent events were recorded descriptively for safety assessment.

Observation Indicators and Detection Methods

The core exposure of interest was 24-hour UGE measured at the 3-month visit after initiation of SGLT2 inhibitor-containing therapy. This was a single predefined 24-hour urine collection rather than the average of repeated quantitative collections. The month-3 time point was selected because it allowed early treatment adaptation, assessment of medication tolerance, and stabilization of the glucosuric response after SGLT2 inhibitor initiation. Patients were required to remain on a stable SGLT2 inhibitor-containing regimen before the 3-month UGE assessment whenever clinically feasible. If dose adjustment or temporary interruption occurred before month 3, exposure classification was based on the regimen status documented at the time of the 24-hour urine collection, and such information was considered during sensitivity assessment. Hydration status, renal function, medication adherence, and completeness of urine collection were reviewed at the exposure visit to reduce measurement error. UGE was quantified by the hexokinase method using a standardized biochemical analyzer. Patients were categorized into quartiles according to the 3-month UGE distribution. Baseline clinical covariates included age, sex, body mass index (BMI), diabetes duration, estimated glomerular filtration rate (eGFR), glycated hemoglobin (HbA1c), prior infection history, menopausal status where applicable, concomitant medications, and major cardiometabolic comorbidities.

Urinary immune-cell subsets were assessed by flow cytometry, soluble immune mediators and antimicrobial peptides were quantified using standardized immunoassays, and targeted urinary metabolomics was performed with a validated ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) platform. All specimens were processed according to standard operating procedures in a central laboratory. To reduce preanalytical variability, sample collection time, storage conditions, and assay batch management were standardized throughout the study.

Statistical Analysis

Analyses were performed using R 4.3.0 and Statistical Package for Social Sciences (SPSS) 26.0 (IBM, Armonk, NY, USA). Continuous variables are presented as mean±SD or median (IQR), as appropriate, and categorical variables as n (%). Between-group comparisons used analysis of variance, Kruskal-Wallis tests, χ^2 tests, or Fisher exact tests as appropriate. For endpoint comparisons with small cell counts, Fisher exact testing was used, or the results were interpreted as exploratory because of limited statistical power.

The primary exposure was the predefined 3-month 24-hour UGE quartile. Time-to-event associations between UGE and the first pathogen-confirmed UGTI were assessed using Cox proportional hazards models with sequential multivariable adjustment. Model 1 was unadjusted. Model 2 adjusted for demographic variables, including age and sex. Model 3 further adjusted for clinically relevant variables, including BMI, diabetes duration, baseline HbA1c, eGFR, prior infection history, major comorbidities, and concomitant glucose-lowering therapy. Specific SGLT2 inhibitor

agent and metformin-containing regimen were considered in sensitivity analyses. Because only 39 primary endpoint events occurred, the number of covariates in multivariable models was restricted to avoid excessive overfitting. The events-per-variable issue was considered when interpreting adjusted estimates.

The proportional hazards assumption was evaluated using Schoenfeld residuals and graphical inspection. Restricted cubic spline analysis was used to explore potential non-linearity, with knots placed at conventional percentiles of the UGE distribution. Exploratory mediation analysis was performed to evaluate whether UGE statistically mediated the association between glycemic change and UGTI risk, under the assumptions of no unmeasured exposure-mediator, mediator-outcome, and exposure-outcome confounding. Biomarker and metabolomics analyses were considered exploratory; correction for multiple comparisons was performed where applicable, and unadjusted findings were interpreted cautiously. Weighted gene co-expression network analysis (WGCNA) was used as an exploratory systems-biology approach to identify immune-metabolic co-expression modules, and the results were interpreted as hypothesis-generating rather than internally validated mechanistic proof.

Missing key exposure data were not imputed because UGE was the main exposure variable. For covariates with limited missingness, complete-case analysis was used. Sensitivity analyses examined the robustness of the main findings after excluding patients with previous UGTI, frequent antibiotic exposure during follow-up, reduced renal function, or unstable SGLT2 inhibitor use before the 3-month exposure assessment. Given the observational design and limited number of endpoint events, mediation and immune-metabolic analyses were interpreted as supportive and hypothesis-generating rather than definitive evidence of causality.

RESULTS

Enrollment of Study Subjects

A total of 412 consecutive T2DM patients who initiated SGLT2 inhibitor-containing therapy were screened. After eligibility assessment and the 3-month exposure ascertainment visit, 380 participants were included in the final analytic cohort and were classified into 4 equal UGE quartiles, with 95 patients in each group. The 32 patients not included in the final analytic cohort were excluded because of early discontinuation of SGLT2 inhibitor-containing therapy for reasons unrelated to infection, loss to follow-up before exposure classification, incomplete 24-hour urine collection or missing key exposure data, withdrawal of consent, or major protocol deviations. The overall attrition rate before final exposure classification was 7.77%. Treatment discontinuation due to infection after exposure classification was retained as a secondary outcome rather than used as an exclusion criterion.

Comparison of Baseline Data

There were no statistically significant differences in age, gender, BMI, diabetes duration, proportion of combined hypertension/dyslipidemia/atherosclerotic cardiovascular disease (ASCVD), baseline hypoglycemic regimen, and other data among the 4 groups (all $p>0.05$), indicating comparability between groups. There were significant differences in baseline fasting blood glucose, HbA1c, eGFR, and urinary albumin-to-creatinine ratio (UACR) among the 4 groups (all $p<0.05$), which showed that the higher the UGE, the higher the baseline blood glucose, HbA1c, and eGFR levels, which was consistent with the pharmacokinetic characteristics of SGLT2i urinary glucose excretion (Table 1).

Occurrence of Infection Events During Follow-up

During follow-up, 39 first pathogen-confirmed UGTI events occurred among the 380 analyzed patients, corresponding to a cumulative incidence of 10.26%. The cumulative incidence increased progressively across UGE quartiles, and Kaplan-Meier analysis showed significantly higher event rates in patients with greater urinary glucose exposure. These findings support a graded association between higher UGE and greater subsequent infection risk.

For secondary endpoints, all-cause infection-related hospitalization, severe infection events, and permanent discontinuation of SGLT2 inhibitor-containing therapy attributed to infection also tended to be more frequent in the higher-UGE groups. However, these secondary endpoint analyses involved small event numbers, particularly for severe infection events, and should therefore be interpreted as exploratory rather than definitive between-group evidence (Table 2).

Correlation Analysis Between UGE and UGTI Risk

Univariable Cox regression showed that UGE,

baseline HbA1c, eGFR, previous infection history, female sex, and menopausal status were associated with UGTI risk (all $p<0.05$). After sequential adjustment for demographic, clinical, and treatment-related covariates, higher UGE remained independently associated with a higher hazard of first UGTI, indicating that the observed association was not fully explained by baseline differences across groups.

Restricted cubic spline analysis suggested a significant non-linear positive dose-response relationship between UGE and UGTI risk (non-linearity $p=0.021$). The infection hazard rose more clearly beyond the middle-to-upper range of UGE, implying a possible threshold-like increase in risk rather than a strictly linear pattern (Table 3).

Table 1. Comparison of baseline data of T2DM patients grouped by UGE quartiles.

Indicator	Total cohort (n=380)	Q1 group (n=95)	Q2 group (n=95)	Q3 group (n=95)	Q4 group (n=95)	*p*
Age, y	54.28±9.67	53.85±10.12	54.62±9.34	54.17±9.85	54.48±9.46	0.924
Male, n (%)	212 (55.79)	53 (55.79)	54 (56.84)	52 (54.74)	53 (55.79)	0.998
BMI, kg/m²	25.72±3.16	25.58±3.24	25.81±3.09	25.67±3.21	25.82±3.12	0.947
Diabetes duration, y	6.00 (3.00, 9.00)	5.00 (3.00, 8.00)	6.00 (3.00, 9.00)	6.00 (3.00, 10.00)	6.00 (3.00, 9.00)	0.812
Fasting glucose, mmol/L	9.27±2.14	7.85±1.62	8.76±1.83	9.64±1.95	10.83±2.06	<0.001
HbA1c, %	8.12±1.24	7.35±0.92	7.84±1.03	8.26±1.12	9.03±1.05	<0.001
Hypertension, n (%)	226 (59.47)	54 (56.84)	55 (57.89)	58 (61.05)	59 (62.11)	0.891
Dyslipidemia, n (%)	241 (63.42)	58 (61.05)	59 (62.11)	61 (64.21)	63 (66.32)	0.897
ASCVD, n (%)	89 (23.42)	21 (22.11)	22 (23.16)	23 (24.21)	23 (24.21)	0.989
Prior infection, n (%)	47 (12.37)	11 (11.58)	12 (12.63)	12 (12.63)	12 (12.63)	0.999
eGFR, mL/min/1.73 m²	87.64±18.25	78.52±16.34	83.46±17.21	90.28±17.86	98.30±16.92	<0.001
UACR, mg/g	28.65 (12.34, 87.62)	22.37 (10.25, 68.45)	26.48 (11.36, 76.52)	30.25 (13.42, 92.37)	35.64 (15.23, 102.58)	0.032
Any metformin-containing regimen, n(%)	326 (85.79)	80 (84.21)	81 (85.26)	82 (86.32)	83 (87.37)	0.942

ASCVD: atherosclerotic cardiovascular disease; BMI: body mass index; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; T2DM: type 2 diabetes mellitus; UACR: urinary albumin-to-creatinine ratio; UGE: urinary glucose excretion.

Table 2. Occurrence of endpoint events in groups by UGE quartiles.

Endpoint event	Total cohort (n=380), n (%)	Q1 group (n=95), n (%)	Q2 group (n=95), n (%)	Q3 group (n=95), n (%)	Q4 group (n=95), n (%)	*p*
First UGTI	39 (10.26)	3 (3.16)	7 (7.37)	12 (12.63)	17 (17.89)	0.008
Infection hospitalization	12 (3.16)	1 (1.05)	2 (2.11)	4 (4.21)	5 (5.26)	0.042
Severe infection events	7 (1.84)	0 (0.00)	1 (1.05)	2 (2.11)	4 (4.21)	0.038
Non-urogenital tract infections	16 (4.21)	2 (2.11)	3 (3.16)	4 (4.21)	7 (7.37)	0.154
Permanent treatment discontinuation due to infection	11 (2.89)	0 (0.00)	2 (2.11)	3 (3.16)	6 (6.32)	0.027

p values were calculated using χ^2 tests or Fisher exact tests as appropriate. Because some secondary endpoints had small cell counts, especially severe infection events, these comparisons should be interpreted as exploratory.

UGE: urinary glucose excretion; UGTI: urogenital tract infection.

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Table 3. Multivariate Cox regression analysis results of urogenital tract infection risk.

Variable	Model 1 HR (95% CI)	*p*	Model 2 HR (95% CI)	*p*	Model 3 HR (95% CI)	*p*
UGE grouping (Q1 as reference)	NA	NA	NA	NA	NA	NA
Q2 group	2.37 (0.61–9.19)	0.212	2.29 (0.59–8.89)	0.230	2.15 (0.55–8.37)	0.271
Q3 group	4.21 (1.18–15.01)	0.027	4.05 (1.13–14.49)	0.032	3.87 (1.06–14.12)	0.040
Q4 group	6.14 (1.81–20.84)	0.004	5.98 (1.76–20.32)	0.004	5.92 (1.69–20.74)	0.006
UGE continuous variable (per 10 g/24 h)	1.21 (1.11–1.32)	<0.001	1.19 (1.09–1.30)	<0.001	1.18 (1.08–1.29)	<0.001

Model 1 is the unadjusted univariate model; Model 2 is adjusted for demographic characteristics; Model 3 is further adjusted for disease-related data, laboratory indicators, and concomitant medication.
CI: confidence interval; HR: hazard ratio; UGE: urinary glucose excretion.

Mediation Effect Analysis Results

Using change in HbA1c after initiation of SGLT2 inhibitor-containing therapy as the exposure, UGTI as the outcome, and UGE as the mediator, exploratory mediation analysis suggested that UGE statistically accounted for part of the association between glycemic improvement and infection risk after multivariable adjustment. Because mediation analysis in observational data relies on strong assumptions and is sensitive to residual confounding and model specification, this result should be interpreted as supportive but not conclusive evidence of a possible mediating pathway.

When UGE was modeled together with selected urinary immune markers, the pattern linking higher UGE, lower macrophage M1/M2 ratio, lower β-defensin 2, and higher UGTI risk appeared statistically plausible. This chain-mediation result is best regarded as a hypothesis-generating observation rather than proof of a complete biological mechanism.

Association Between UGE and Urogenital Immune-Metabolic Crosstalk

In follow-up samples obtained at the 3-month visit, urinary immune indices differed significantly across UGE groups (Table 4). With increasing UGE, urinary neutrophil proportion increased, whereas macrophage M1/M2 ratio, $CD4^+/CD8^+$ T-cell ratio, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, β-defensin 2, and LL-37 decreased, while IL-10 increased. Overall, higher UGE was associated with a urinary immune profile suggestive of altered local antimicrobial defense. These findings should be interpreted as associative biomarker patterns rather than direct evidence that urinary glucose caused mucosal immune suppression.

Targeted urinary metabolomics identified 22 differential metabolites across groups (VIP > 1, $p<0.05$), mainly enriched in glycolysis- and tricarboxylic acid cycle-related pathways. Compared with the Q1 group, patients in the higher UGE groups showed lower levels of pyruvate, lactate, and citrate, suggesting altered local metabolic activity in parallel with the immune changes observed above.

WGCNA identified a core immune-metabolic co-expression module that correlated with both UGE level and infection outcome ($r = 0.72$, $p<0.001$). Hub features included the macrophage M1/M2 ratio, β-defensin 2, pyruvate, and lactate. These findings support internal coherence of the measured biomarkers, although external validation is still required.

Sensitivity Analysis

Sensitivity analyses yielded results broadly consistent with the primary analysis after excluding patients with previous UGTI, frequent antibiotic use during follow-up, reduced renal function, or after redefining exposure using time-weighted average urinary glucose. The persistence of the main signal across these analyses supports the robustness of the observed association, but does not eliminate the possibility of residual confounding.

Table 4. Comparison of urinary immune indexes in groups by UGE quartiles.

Indicator	Q1 group (n=95)	Q2 group (n=95)	Q3 group (n=95)	Q4 group (n=95)	*p*
Neutrophils, %	12.35 (8.24, 16.52)	15.62 (10.35, 20.48)	20.47 (14.26, 26.58)	26.84 (18.62, 34.57)	<0.001
M1/M2 ratio	1.86 (1.42, 2.35)	1.52 (1.15, 1.94)	1.18 (0.85, 1.56)	0.76 (0.52, 1.08)	<0.001
$CD4^+/CD8^+$ T cell ratio	1.64 (1.25, 2.08)	1.48 (1.12, 1.86)	1.25 (0.94, 1.58)	0.98 (0.72, 1.32)	<0.001
IL-1β, pg/mL	38.62 (24.58, 52.64)	32.45 (20.36, 45.72)	25.37 (16.24, 36.58)	18.42 (10.25, 28.64)	<0.001
TNF-α, pg/mL	42.58 (28.64, 58.62)	36.47 (24.52, 50.37)	28.64 (18.45, 40.26)	20.35 (12.24, 30.48)	<0.001
β-defensin 2, pg/mL	248.64 (186.52, 324.58)	202.47 (152.36, 268.42)	156.35 (112.24, 208.64)	98.26 (64.15, 142.58)	<0.001
LL-37, pg/mL	186.52 (136.42, 248.64)	154.47 (108.36, 202.58)	122.35 (82.24, 164.42)	78.26 (48.15, 118.64)	<0.001
IL-10, pg/mL	12.45 (8.24, 16.58)	16.52 (10.36, 22.48)	22.37 (14.25, 30.62)	28.64 (18.42, 38.57)	<0.001

[a]All urinary immune indices were measured at the 3-month visit, the same visit at which 24-hour UGE was assessed. Values are presented as median (P25, P75).

CD: cluster of differentiation; IL: interleukin; LL-37: cathelicidin antimicrobial peptide; M1: classically activated macrophage; M2: alternatively activated macrophage; TNF-α: tumor necrosis factor α; UGE: urinary glucose excretion.

DISCUSSION

In this prospective cohort study, higher 24-hour UGE measured after initiation of SGLT2 inhibitor-containing therapy was consistently associated with a greater risk of subsequent pathogen-confirmed UGTI in patients with T2DM. The data also showed coordinated differences in urinary immune and metabolic markers across UGE quartiles. Taken together, these findings support UGE as a clinically relevant exposure marker and possible mediator candidate, but they do not establish direct causality or definitive immune-metabolic mechanisms.

Interpreting the Clinical Association between SGLT2 Inhibitor-containing Therapy, Glycemic Improvement, and Infection Risk

Since the launch of SGLT2i, the contradictory phenomenon that it "lowers blood glucose yet increases infection risk" has always been the focus of clinical and academic attention. The traditional theory holds that hyperglycemia is the core driving factor for the increased infection susceptibility of diabetic patients, and effective glucose lowering should reduce the infection risk. However, the clinical data of SGLT2i are completely contrary to this theory.[3] Previous studies mostly attributed this paradox to confounding factors, such as drug class effect, gender, renal function, or simply attributed it to the nutritional support effect of urinary glucose on pathogens, but none of them can explain the core contradictory phenomena in clinical practice.

The mediation analysis is consistent with this interpretation, because a substantial portion of the association between glycemic change and infection outcome appeared to operate through UGE. Nevertheless, this should not be overstated. Mediation estimates derived from observational data depend on model assumptions and adequate control of confounding. The current study therefore supports, but does not prove, the view that urinary glucose is an intermediate clinical pathway linking SGLT2 inhibitor-containing therapy to infection susceptibility in some patients.

From a translational perspective, the results suggest that urinary glucose burden deserves attention as a measurable phenotype rather than a simple pharmacodynamic epiphenomenon. This distinction matters because it opens the possibility of identifying subgroups at higher infection risk despite otherwise favorable metabolic response to treatment. The findings also underscore the importance of integrating local exposure markers into future safety research on SGLT2 inhibitor-containing therapy.

Immune-metabolic Crosstalk as a Plausible Explanatory Framework

The second major contribution of the study is the integration of urinary immune and metabolic indicators into the analysis of infection risk. Higher UGE was

accompanied by a lower macrophage M1/M2 ratio, reduced β-defensin 2 and IL-8, and coordinated shifts in glycolysis- and TCA cycle-related metabolites. These patterns are compatible with the hypothesis that excess glucosuria may be linked to a less effective local antimicrobial state. Importantly, the data do not establish direct molecular inhibition by glucose itself; rather, they indicate an association between greater urinary glucose exposure and a biomarker constellation suggestive of impaired host defense.

This interpretation fits with the broader literature on immune-metabolic coupling. Effective innate immune responses require adequate metabolic reprogramming, and disturbances in the local nutrient environment can alter macrophage phenotype, cytokine secretion, and epithelial defense activity. Within the urinary tract, such changes could plausibly influence how patients respond to microbial colonization. The present clinical dataset cannot resolve cell-specific mechanisms, but it does provide a rational translational bridge between clinical epidemiology and experimental work.

The exploratory chain-mediation model further suggests that immune-metabolic markers may partly lie along the pathway connecting UGE to infection events. Because endpoint numbers were limited and the biomarker network was measured in a single-center cohort, these analyses should be viewed as hypothesis-generating. Replication in independent cohorts and mechanistic validation in experimental systems will be essential before firm biological claims can be made.

The findings may also help explain inter-individual heterogeneity in SGLT2i-associated infection risk. Patients with similar levels of systemic glucose control may differ in urinary glucose exposure, mucosal immune resilience, urinary flow patterns, hormonal milieu, prior colonization status, and hygiene-related factors. A multifactorial model is therefore more realistic than any single-cause explanation. UGE may be one important upstream driver within this broader susceptibility network.

Clinical Implications

It is important to emphasize that the present findings should not be interpreted as discouraging the use of SGLT2 inhibitor-containing therapy. These agents remain guideline-supported treatments with well-established cardiovascular and renal benefits in appropriately selected patients.[7] Most urogenital infections associated with SGLT2 inhibitor use are mild to moderate and clinically manageable. Therefore, the clinical value of the present findings lies in improving risk stratification, patient education, and early recognition of infection rather than limiting access to therapies with proven cardio-renal benefit.

Several potential clinical implications emerge from this study. First, 24-hour UGE may be useful as a practical risk-enrichment marker in patients starting SGLT2 inhibitor-containing therapy, particularly in those with a prior infection history, female sex, postmenopausal status, or other predisposing factors. Second, the results reinforce the importance of early education on genital and urinary symptoms, hydration, hygiene, and prompt evaluation of suspected infection. Third, the data suggest that infection prevention during SGLT2 inhibitor-containing therapy may eventually benefit from a stratified approach that considers both systemic metabolic status and local urinary tract vulnerability. These implications remain provisional and should be prospectively tested before being incorporated into routine protocols.

Limitations and Future Directions

This study has several limitations. First, it was a single-center observational cohort, and residual confounding cannot be excluded despite multivariable adjustment and sensitivity analyses. Several factors that may influence urogenital infection risk, such as detailed sexual activity, hygiene practices, circumcision status in men, menopausal hormone use, antibiotic exposure patterns, and glycemic variability, were not fully captured. Therefore, the causal interpretation of UGE should be cautious. Second, UGE was measured using a single predefined 24-hour urine collection at the 3-month visit. Although this time point was selected to reflect early stabilized glucosuric exposure after treatment initiation, a single measurement may not fully represent long-term urinary glucose burden. Repeated quantitative UGE assessment and time-updated exposure modeling would strengthen future studies. Third, the 3-month exposure ascertainment window may have introduced selection bias because patients who discontinued therapy or failed to complete exposure assessment before that time were not included in the final analytic cohort. Fourth, only 39 primary endpoint events occurred, limiting model complexity, subgroup analyses, mediation estimates, and high-dimensional biomarker modeling. The possibility of model

overfitting should therefore be considered. Fifth, the immune-metabolic findings were associative and hypothesis-generating. Lower M1/M2 ratio, altered cytokines, antimicrobial peptide changes, and differences in urinary metabolites do not prove that urinary glucose directly suppresses mucosal immunity. Sixth, the primary endpoint combined bacterial urinary tract infection and genital infection into a composite UGTI outcome, which may obscure clinically meaningful differences between infection types.

Future studies should include multicenter prospective validation, repeated UGE measurement, time-updated exposure models, sex-specific and infection-type-specific analyses, microbiome and epithelial-barrier assessment, and mechanistic experiments testing how glucosuria influences immune-cell metabolism and antimicrobial defense in the urogenital tract.

In summary, this prospective cohort study found that higher UGE after initiation of SGLT2 inhibitor-containing therapy was associated with a higher subsequent risk of pathogen-confirmed UGTI in patients with T2DM. The findings further suggest that unfavorable changes in urinary immune and metabolic markers may accompany this association. The work therefore provides clinical evidence supporting urinary glucose as a plausible mediator candidate and risk marker, while emphasizing the need for external validation and mechanistic confirmation.

STATEMENT OF ETHICS

The study protocol was reviewed and approved by the institutional ethics committee of the study hospital, and all participants provided written informed consent before enrollment. Study procedures conformed to the principles of the Declaration of Helsinki. Biological specimens were collected only for protocol-specified analyses, and all data were de-identified before statistical analysis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Genitsaridi I, Salpea P, Salim A, Sajjadi SF, Tomic D, James S, et al. 11th edition of the IDF Diabetes Atlas: global, regional, and national diabetes prevalence estimates for 2024 and projections for 2050. The Lancet. Diabetes & Endocrinology. 2026;14(2):149-56.
2. Critchley JA, Carey IM, Harris T, DeWilde S, Hosking FJ, Cook DG. Glycemic Control and Risk of Infections Among People With Type 1 or Type 2 Diabetes in a Large Primary Care Cohort Study. Diabetes Care. 2018;41(10):2127-35.
3. Pishdad R, Auwaerter PG, Kalyani RR. Diabetes, SGLT-2 Inhibitors, and Urinary Tract Infection: a Review. Curr Diab Rep. 2024 May;24(5):108-117.
4. Jafar N, Edriss H, Nugent K. The Effect of Short-Term Hyperglycemia on the Innate Immune System. The American Journal of the Medical Sciences. 2016;351(2):201-11.
5. Hodgson K, Morris J, Bridson T, Govan B, Rush C, Ketheesan N. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. Immunology. 2015;144(2):171-85.
6. Vallon V. The mechanisms and therapeutic potential of SGLT2 inhibitors in diabetes mellitus. Annu Rev Med. 2015;66:255-70.
7. Qaseem A, Obley AJ, Shamliyan T, Hicks LA, Harrod CS, Crandall CJ, et al. Newer Pharmacologic Treatments in Adults With Type 2 Diabetes: A Clinical Guideline From the American College of Physicians. Ann Intern Med. 2024;177(5):658-66.
8. Puckrin R, Saltiel M, Reynier P, Azoulay L, Yu OHY, Filion KB. SGLT-2 inhibitors and the risk of infections: a systematic review and meta-analysis of randomized controlled trials. Acta Diabetol. 2018;55(5):503-14.

9. Johnsson KM, Ptaszynska A, Schmitz B, Sugg J, Parikh SJ, List JF. Vulvovaginitis and balanitis in patients with diabetes treated with dapagliflozin. J Diabetes Complicat. 2013;27(5):479-84.
10. Silverii GA, Dicembrini I, Monami M, Mannucci E. Fournier's gangrene and sodium-glucose co-transporter-2 inhibitors: A meta-analysis of randomized controlled trials. Diabetes, Obesity & Metabolism. 2020;22(2):272-5.
11. Riksen NP, Netea MG. Immunometabolic control of trained immunity. Mol Aspects Med. 2021;77(100897).
12. Alba-Loureiro TC, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, et al. Neutrophil function and metabolism in individuals with diabetes mellitus. Braz J Med Biol Res. 2007;40(8):1037-44.
13. Sha S, Polidori D, Farrell K, Ghosh A, Natarajan J, Vaccaro N, et al. Pharmacodynamic differences between canagliflozin and dapagliflozin: results of a randomized, double-blind, crossover study. Diabetes, Obesity & Metabolism. 2015;17(2):188-97.