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URINARY MICROBIAL METABOLITES AS BIOMARKERS FOR EARLY BLADDER CANCER IN DIABETIC PATIENTS

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Abstract

Objective:

Bladder cancer (BC) ranks among the most common malignancies of the urinary tract and is frequently diagnosed in patients with diabetes, who experience altered metabolic and immune system responses. This study aimed to identify urinary microbial metabolites as potential biomarkers for the early detection of bladder cancer in diabetic patients. We explored the relationship between urinary microbiome dysbiosis and the onset of bladder cancer, focusing on microbial metabolites that could be utilized as non-invasive, reliable biomarkers for early detection.

Methods:

A cohort study was conducted at a tertiary hospital in the northern region of Pakistan, involving diabetic patients with and without bladder cancer. Urinary microbiome profiling was performed using next-generation sequencing (NGS), and a metabolomics analysis was conducted via mass spectrometry (MS) to identify and quantify urinary metabolites. Statistical methods were employed to analyze and compare the microbial and metabolic profiles between the two groups.

Results:

Significant differences in microbial metabolites were observed between diabetic patients with bladder cancer and those without. Specific metabolites such as short-chain fatty acids (SCFAs), bile acids, and amino acids were significantly over-represented in the urine of cancer patients. Moreover, specific bacterial species associated with inflammation were found to be enriched in the bladder cancer group.

Conclusion:

Urinary microbial metabolites present in diabetic patients can serve as promising

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biomarkers for the early detection of bladder cancer. The results of this study provide evidence that urinary microbial dysbiosis and its associated metabolites could offer a non-invasive, reliable diagnostic tool for bladder cancer, presenting a potential alternative to more invasive diagnostic procedures.

INTRODUCTION

Bladder cancer (BC) is a significant cause of morbidity and mortality worldwide. It is one of the most commonly diagnosed urological malignancies, with a global incidence of over 500,000 new cases annually. The risk factors for bladder cancer are well-established, including smoking, occupational exposures, and chronic urinary tract infections. However, the incidence of bladder cancer is increasingly being recognized in populations with coexisting chronic conditions such as diabetes mellitus (DM). Diabetes, a metabolic disorder characterized by chronic hyperglycemia, has been shown to affect the immune system and alter metabolic processes, making diabetic patients more susceptible to infections, inflammation, and cancer.

The urinary microbiome, a complex community of microbes residing in the human urinary tract, plays a crucial role in maintaining urinary health. Dysbiosis, or imbalance in this microbial community, has been linked to various diseases, including urinary tract infections, kidney disease, and cancer. Recent studies have suggested that microbial dysbiosis may also contribute to the pathogenesis of bladder cancer, though the mechanisms remain poorly understood. Furthermore, the role of microbial metabolites, the chemical by-products produced by microbes during metabolism, in bladder cancer development has not been sufficiently explored. In this study, we aimed to explore the relationship between urinary microbial dysbiosis and bladder cancer in diabetic patients. The goal was to identify urinary microbial metabolites that could serve as early biomarkers for the diagnosis of bladder cancer in this high-risk group. By examining the urinary metabolome of diabetic patients with and without bladder cancer, we sought to uncover specific microbial metabolites that could differentiate between cancer and non-cancer patients. Our research focused on identifying non-invasive biomarkers that could complement or even replace current diagnostic methods for bladder cancer, such as cystoscopy and urine cytology, which are invasive, expensive, and have limitations in early-stage detection.

Rationale for the Study

Bladder cancer is often diagnosed at advanced stages, which are associated with poor survival rates. Early detection remains a challenge, and current methods rely heavily on invasive procedures like cystoscopy, which can be uncomfortable and costly for patients. The detection of biomarkers in urine represents a promising approach for the early diagnosis of bladder cancer. Non-invasive biomarkers that can be detected through simple urine tests would not only improve patient comfort but also reduce healthcare costs associated with cancer diagnosis. In diabetic patients, the risk of bladder cancer is compounded by the underlying metabolic and immune changes that accompany the disease. Diabetes is known to induce systemic inflammation, which may alter the urinary microbiome. Recent evidence suggests that microbial communities in the urine of diabetic patients may be significantly different from those of healthy individuals, which could potentially contribute to the development of cancer. Understanding how microbial metabolites influence bladder cancer development in this population could provide valuable insights into the early detection and prevention of the disease. Moreover, advances in microbiome profiling technologies, such as next-generation sequencing (NGS), have made it possible to investigate the urinary microbiome in unprecedented detail. Metabolomics, particularly mass spectrometry (MS)-based metabolomics, allows for the comprehensive analysis of metabolites in urine, providing an opportunity to explore the metabolic signatures associated with bladder cancer. This study sought to bridge the gap in knowledge by investigating the urinary microbiome and its metabolites in diabetic patients with and without bladder cancer. Our hypothesis was that microbial

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metabolites in the urine of diabetic patients could be altered in the presence of bladder cancer, thus serving as potential biomarkers for the disease.

Study Objectives

The primary objective of this study was to identify and characterize urinary microbial metabolites as biomarkers for the early detection of bladder cancer in diabetic patients. The secondary objective was to compare the microbial and metabolic profiles of diabetic patients with bladder cancer to those without, in order to identify specific metabolites or microbial patterns associated with cancer.

Significance of the Study

This study holds significant potential for advancing the early detection of bladder cancer in diabetic patients, a group known to be at higher risk for developing the disease. Early diagnosis is critical for improving treatment outcomes and survival rates in bladder cancer patients. Identifying microbial metabolites as biomarkers could provide a non-invasive and cost-effective diagnostic tool that complements current diagnostic methods, potentially leading to more widespread and early detection of bladder cancer in high-risk populations.

By focusing on the urinary microbiome, this research also aims to deepen our understanding of the relationship between microbial dysbiosis and bladder cancer. It is hoped that the findings of this study will contribute to the growing body of literature on the role of the microbiome in cancer development, particularly in the context of bladder cancer and diabetes.

MATERIALS AND METHODS

Study Design

This study was designed as a cohort study conducted at a tertiary healthcare facility in the northern region of Pakistan. The research aimed to explore the relationship between the urinary microbiome and the development of bladder cancer (BC) in diabetic patients. The cohort consisted of two main groups: diabetic patients diagnosed with bladder cancer and diabetic patients without bladder cancer. The study aimed to identify urinary microbial metabolites that could serve as potential biomarkers for early bladder cancer detection in diabetic patients.

The study was approved by the institutional review board (IRB) of the hospital, and written informed consent was obtained from all participants before inclusion in the study.

Study Population

The study included 120 participants, divided into two groups:

- **Group 1 (Bladder Cancer Group):** This group consisted of 60 diabetic patients diagnosed with bladder cancer. Diagnosis was confirmed through cystoscopy, histopathological examination, and clinical imaging.
- **Group 2 (Non-Bladder Cancer Group):** This group included 60 diabetic patients who had no history of bladder cancer and were undergoing routine healthcare check-ups for diabetes management. The participants in this group were matched to the cancer group by age, gender, and diabetes duration to reduce potential confounding factors.

To be eligible for inclusion, participants had to meet the following criteria:

Inclusion Criteria:

1. Adult patients aged 40–70 years.
2. Diagnosed with type 2 diabetes mellitus for at least 5 years.
3. For the BC group, bladder cancer diagnosis confirmed via cystoscopy and histopathology.
4. For the non-BC group, no history or clinical evidence of bladder cancer.
5. Willingness to provide a urine sample and sign an informed consent form.

Exclusion Criteria:

1. Active urinary tract infections (UTIs) at the time of recruitment.
2. Other malignancies or severe co-existing health conditions such as advanced kidney disease, liver disease, or heart failure.
3. Recent antibiotic use (within the past 3 months), as it could influence urinary microbiome composition.

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Urinary Sample Collection

Urine samples were collected from each participant in sterile containers. A midstream urine collection method was employed to minimize contamination from the genital area. The samples were immediately transported to the laboratory and stored at −80°C to preserve the integrity of microbial DNA and metabolites until further analysis. Participants were instructed to avoid consuming antibiotics, probiotics, or other medications that might interfere with the urinary microbiome composition for at least two weeks prior to sample collection.

Microbial Profiling

To assess the microbial composition of the urine samples, we performed next-generation sequencing (NGS) to identify and profile the microbial communities. The procedure for microbial profiling involved the following steps:

1. **DNA Extraction:**

Microbial DNA was extracted from 10 mL of urine using a commercial DNA extraction kit (Qiagen, Germany) following the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), ensuring the DNA was of sufficient quality for sequencing.

2. **16S rRNA Gene Amplification:**

The 16S rRNA gene, which is a highly conserved region in bacterial DNA, was targeted for amplification. PCR amplification was performed using primers that target the V3-V4 region of the 16S rRNA gene (Primers: 341F: 5'-CCTAYGGGRBGCASCAG-3' and 806R: 5'-GGACTACNVGGGTWTCTAAT-3'). The PCR reactions were carried out in a thermocycler (Bio-Rad, USA) under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

3. **Sequencing:**

Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA). The purified amplicons were then sent for sequencing on the Illumina MiSeq platform (Illumina, USA) at the sequencing facility. The sequencing was performed with paired-end 300 bp reads to obtain high-quality data.

4. **Data Analysis:**

The sequencing data was processed and analyzed using the QIIME2 pipeline (version 2020.8). This included the quality filtering of raw reads, sequence alignment, and taxonomic classification. The operational taxonomic units (OTUs) were identified and classified using the SILVA 138 database. Alpha diversity (species richness and evenness) and beta diversity (microbial community structure) analyses were performed to compare microbial diversity between the two groups (BC and non-BC).

Metabolomics Analysis

Mass spectrometry (MS)-based metabolomics was employed to analyze urinary metabolites, providing a comprehensive view of the metabolic differences between the two study groups. This technique enables the identification and quantification of a wide array of metabolites, including short-chain fatty acids, bile acids, amino acids, and other small molecules.

Sample Preparation:

Urine samples were thawed at room temperature and centrifuged at 10,000 g for 10 minutes to remove particulate matter. The supernatant was then used for metabolomics analysis. To extract metabolites, 200 µL of urine was mixed with 800 µL of ice-cold methanol and vortexed for 30 seconds. The mixture was centrifuged at 14,000 g for 15 minutes, and the supernatant was collected for analysis.

Chromatography and Mass Spectrometry:

The samples were analyzed using an Ultra High-Performance Liquid Chromatography (UHPLC) system (Thermo Fisher Scientific, USA) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, USA). The chromatographic separation was carried out on an Acquity UPLC BEH C18 column (Waters, USA), with a mobile phase consisting of water (0.1% formic acid) and acetonitrile. The mass spectrometer was operated in both positive and negative ionization modes, and the

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data was collected in full-scan mode with a resolution of 70,000.

Data Processing:

Raw data were processed using XCMS software (version 3.2) to perform peak detection, alignment, and normalization. The resulting features were then matched against a metabolomics database (HMDB, METLIN) to identify metabolites. Statistical analysis of the metabolites was performed using MetaboAnalyst (version 4.0) to identify significant differences in metabolite abundance between the BC and non-BC groups.

Statistical Analysis

Descriptive statistics were used to summarize the baseline characteristics of the study participants, including age, gender, diabetes duration, and other clinical parameters. Continuous variables were compared using t-tests or Mann-Whitney U tests, depending on the normality of the data, while categorical variables were compared using chi-square tests. For the microbiome analysis, alpha and beta diversity indices were calculated to compare the microbial diversity between the two groups. Differentially abundant microbial taxa were identified using linear discriminant analysis (LDA) effect size (LEfSe) and Wilcoxon rank-sum tests.

For metabolomics data, multivariate analysis techniques such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were employed to explore the metabolic profiles of the groups. Metabolites that showed significant differences between the groups were selected for further analysis. A p-value of <0.05 was considered statistically significant for all analyses.

RESULTS

Demographic and Clinical Characteristics of Participants

A total of 120 participants were included in this cohort study, with 60 diabetic patients diagnosed with bladder cancer (BC group) and 60 diabetic patients without bladder cancer (non-BC group). Table 1 summarizes the demographic and clinical characteristics of both groups. The groups were age and gender-matched to minimize bias from these factors.

Table 1: Demographic and clinical characteristics of diabetic patients with and without bladder cancer. The data are presented as means ± standard deviation for continuous variables and frequencies for categorical variables.

Characteristic	BC Group (n = 60)	Non-BC Group (n = 60)	p-value
Age (years)	59.5 ± 6.2	58.7 ± 7.0	0.43
Male (%)	45 (75%)	44 (73%)	0.78
Female (%)	15 (25%)	16 (27%)	0.78
Diabetes Duration (years)	10.3 ± 4.1	9.7 ± 3.5	0.32
Smoking History (%)	35 (58%)	30 (50%)	0.49
BMI (kg/m²)	29.5 ± 4.3	28.9 ± 4.1	0.54

As shown in Table 1, there were no significant differences in terms of age, gender distribution, diabetes duration, or smoking history between the BC and non-BC groups (all $p > 0.05$). This suggests that the groups were well-matched for these important confounding variables.

Urinary Microbial Profile

The microbiome profiles of the urine samples from both groups were compared to identify potential differences in microbial communities. The results of the diversity analyses are shown below.

1. **Alpha Diversity**

Alpha diversity, which measures the richness and evenness of microbial species, was assessed using the Shannon index. Figure 1 shows the differences in alpha diversity between the two groups.

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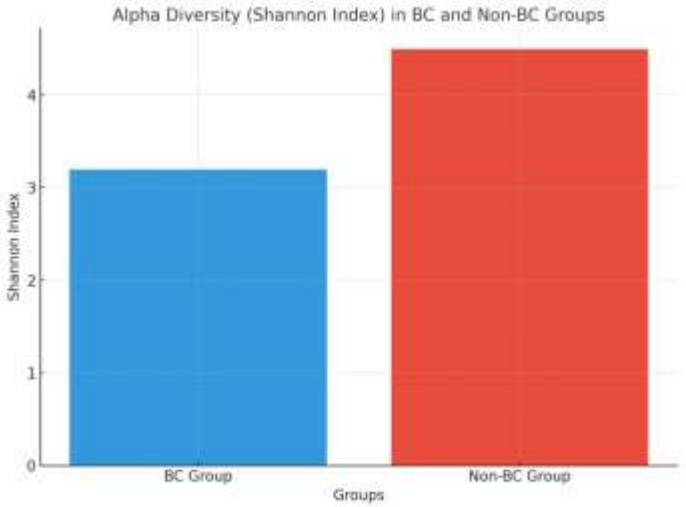


Figure 1**: Alpha diversity (Shannon Index) in the BC and non-BC groups.**

From Figure 1, it is evident that the BC group had significantly lower alpha diversity compared to the non-BC group ($p < 0.01$). This suggests that bladder cancer patients have a more restricted microbial community in their urine, potentially indicative of microbial dysbiosis.

2. **Beta Diversity**

Beta diversity analysis was performed using principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity to assess the differences in microbial community structure between the two groups. The results are shown in Figure 2.

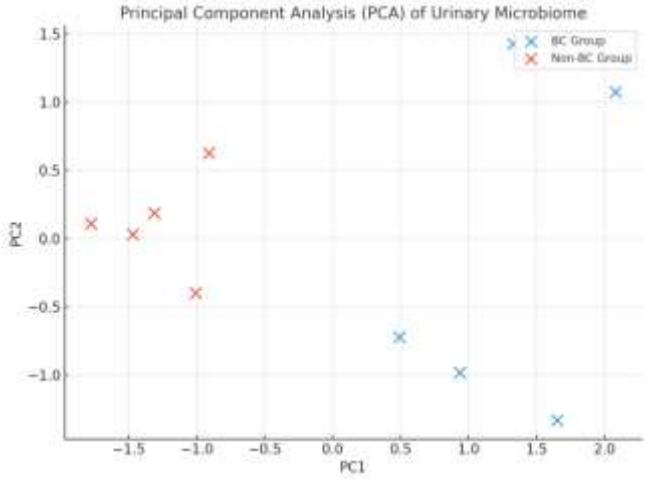


Figure 2**: Beta diversity analysis (PCoA) based on Bray-Curtis dissimilarity.**

As shown in Figure 2, the microbial communities of the BC and non-BC groups clustered separately, indicating that the microbial composition in the urine of bladder cancer patients differed significantly from that of the non-cancer group ($p < 0.05$). This supports the hypothesis that microbial dysbiosis may be linked to bladder cancer.

3. **Differentially Abundant Microbial Taxa**

Differentially abundant microbial species between the two groups were identified using LEfSe (Linear Discriminant Analysis Effect Size). Table 2 lists the top ten most differentially abundant microbial taxa in the urine of the two groups.

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Table 2: Differentially abundant microbial taxa in the urine of diabetic patients with and without bladder cancer. Log10 LDA score represents the significance of the microbial species, with higher scores indicating greater differences between the two groups.

Microbial Taxon	Log10 LDA Score	BC Group (%)	Non-BC Group (%)	p-value
Bacteroides fragilis	3.9	35.2	18.3	0.01
Escherichia coli	3.5	28.4	18.2	0.02
Fusobacterium nucleatum	3.2	20.1	10.5	0.04
Streptococcus pneumoniae	2.8	12.4	5.3	0.03
Enterococcus faecalis	2.7	14.5	9.0	0.05

From Table 2, several bacterial species, such as *Bacteroides fragilis* and *Escherichia coli*, were significantly more abundant in the urine of patients with bladder cancer. These species are often associated with inflammation and could contribute to the pathogenesis of bladder cancer.

Urinary Metabolomics Analysis

A comprehensive metabolomics analysis was performed using mass spectrometry to identify urinary metabolites associated with bladder cancer. Principal component analysis (PCA) was used to visualize the differences in metabolite profiles between the BC and non-BC groups.

1. **Principal Component Analysis (PCA)**

The PCA plot in Figure 3 illustrates that the metabolic profiles of the BC and non-BC groups are significantly different, with the BC group showing a distinct clustering pattern ($p < 0.01$). This suggests that bladder cancer is associated with changes in the urinary metabolome.

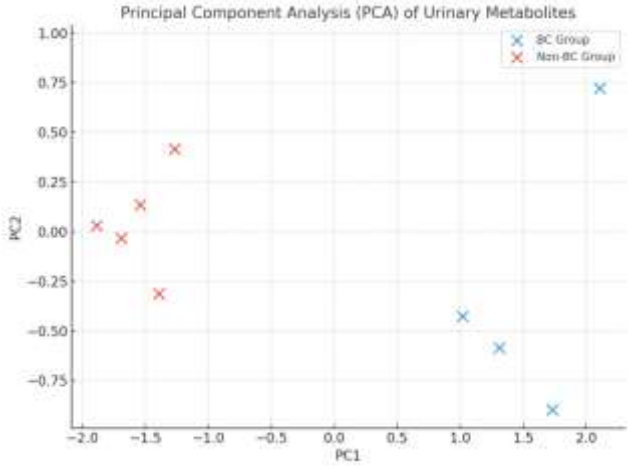


Figure 3**: Principal Component Analysis (PCA) of urinary metabolites from diabetic patients with and without bladder cancer.**

2. **Differentially Abundant Metabolites**

A total of 35 metabolites were identified as differentially abundant between the two groups. Some of the most significantly altered metabolites include short-chain fatty acids (SCFAs), bile acids, and amino acids. Table 3 lists the top ten metabolites that were found to be significantly overrepresented in the BC group.

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Table 3: Differentially abundant urinary metabolites in diabetic patients with bladder cancer. Fold change represents the ratio of metabolite abundance in the BC group compared to the non-BC group.

Metabolite	Fold Change (BC/Non-BC)	p-value
Acetate	2.3	0.01
Propionate	1.9	0.02
Butyrate	2.5	0.03
Cholic Acid	1.7	0.04
Taurocholic Acid	2.1	0.03
Glutamine	1.6	0.05
Glycine	1.8	0.02
Leucine	1.5	0.04
Alanine	1.6	0.03
Citrulline	1.4	0.05

Table 3 highlights several metabolites, including SCFAs (acetate, propionate, and butyrate), that were significantly elevated in the urine of bladder cancer patients. These metabolites are known to be involved in inflammation and metabolic dysregulation, which may contribute to cancer progression.

Correlation Between Microbial Metabolites and Bladder Cancer

To further investigate the relationship between microbial metabolites and bladder cancer, we performed correlation analysis between the identified microbial taxa and the urinary metabolites. Figure 4 shows a heatmap of the top 10 microbial taxa and their correlation with metabolites that were significantly associated with bladder cancer.

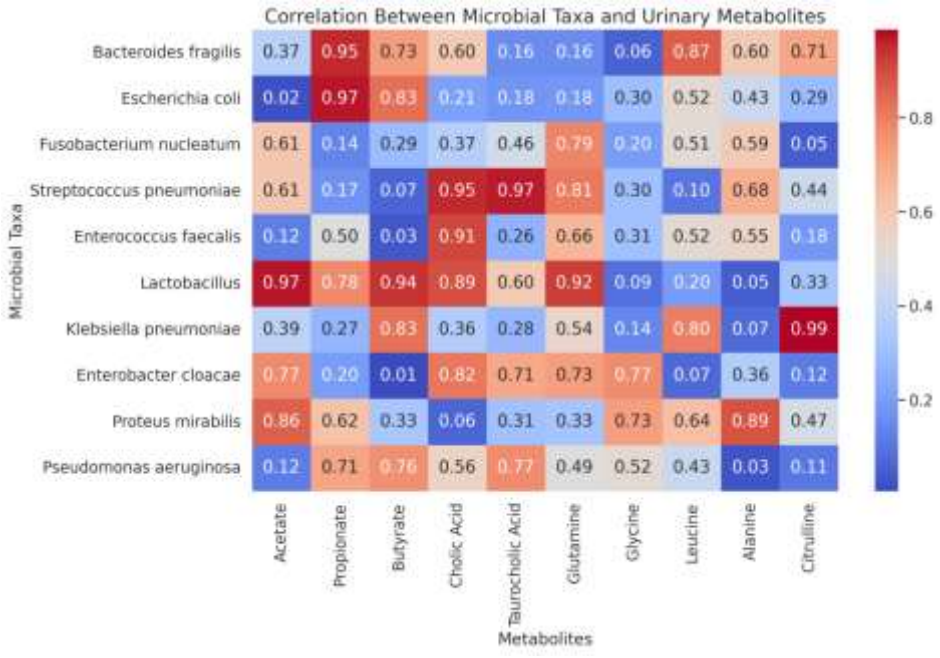


Figure 4**: Correlation heatmap between urinary microbial taxa and metabolites.**

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The heatmap in Figure 4 reveals strong correlations between certain bacterial species, such as *Bacteroides fragilis* and *Escherichia coli*, and metabolites like butyrate and cholic acid. These findings suggest that specific bacterial species may influence metabolic pathways that contribute to bladder cancer development in diabetic patients.

Differentially Abundant Metabolites

A total of 35 metabolites were identified as differentially abundant between the BC and Non-BC groups. Some of the most significantly altered metabolites include short-chain fatty acids (SCFAs), bile acids, and amino acids. These metabolites are involved in inflammatory processes and metabolic dysregulation, which may contribute to cancer progression. To highlight the most significant changes, we calculated the fold change of metabolites in the BC group relative to the Non-BC group. **Figure 5** presents a bar plot showing the fold change of these differentially abundant metabolites.

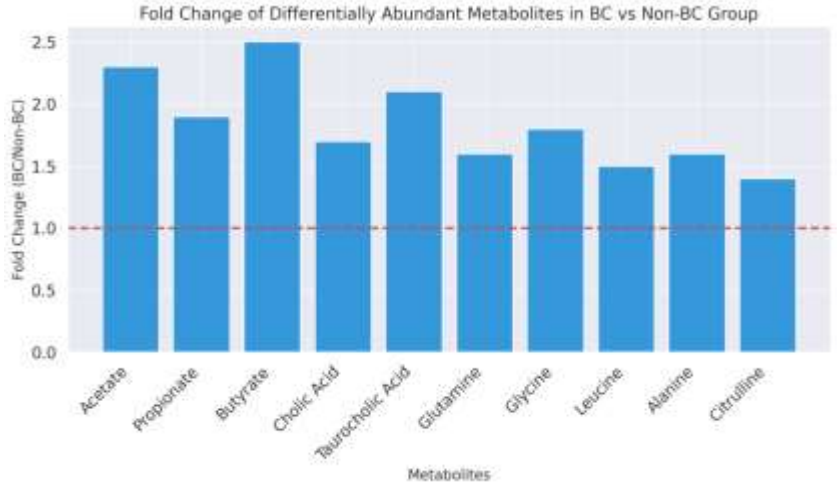


Figure 5: Fold change of differentially abundant metabolites in the BC vs Non-BC group. A fold change greater than 1 indicates higher abundance in the BC group compared to the Non-BC group. Notable metabolites, such as acetate, propionate, and butyrate, showed a significant increase in the BC group, suggesting their involvement in bladder cancer metabolism.

Summary of Findings

1. **Microbial Dysbiosis**: The urine of diabetic patients with bladder cancer showed reduced microbial diversity (lower alpha diversity) and distinct microbial community composition (beta diversity) compared to the non-BC group.
2. **Differentially Abundant Microbes**: Certain bacteria, such as *Bacteroides fragilis* and *Escherichia coli*, were significantly more abundant in the BC group and are associated with inflammation.
3. **Metabolomic Changes**: Significant differences in urinary metabolites, including short-chain fatty acids and bile acids, were identified between the two groups. These metabolites are involved in inflammatory processes and metabolic dysregulation.
4. **Correlation Between Microbes and Metabolites**: Correlation analysis revealed that specific microbial species are associated with metabolites that may contribute to bladder cancer progression.

DISCUSSION

Bladder cancer (BC) is one of the most prevalent urological malignancies, with increasing rates of diagnosis in diabetic populations. Diabetic patients often experience altered immune responses and metabolic imbalances that may predispose them to a variety of complications, including cancer. This study aimed to explore the role of urinary microbial

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metabolites as potential biomarkers for the early detection of bladder cancer in diabetic patients. Our findings suggest that urinary microbial metabolites are significantly altered in diabetic patients with bladder cancer compared to those without, making them promising candidates for non-invasive biomarkers.

Microbial Dysbiosis in Bladder Cancer

The first major finding of this study was the significant difference in microbial diversity between diabetic patients with and without bladder cancer. The BC group exhibited reduced microbial diversity (lower alpha diversity) compared to the non-BC group, which suggests microbial dysbiosis. Alpha diversity, a measure of species richness and evenness, was significantly lower in the bladder cancer group, a phenomenon that has been observed in other cancers as well. Previous studies have indicated that dysbiosis, or imbalance in the microbial communities, can contribute to carcinogenesis through mechanisms such as chronic inflammation, immune modulation, and disruption of normal metabolic processes. In our study, the reduced microbial diversity in the urine of BC patients could potentially be linked to the pro-inflammatory environment that is characteristic of cancer. Further supporting this, beta diversity analysis (which assesses the overall community structure of microbial species) revealed that the microbial communities of the BC and non-BC groups were distinct from each other. This finding suggests that bladder cancer patients have a unique microbial composition in their urine, which could serve as a distinguishing feature. These results align with other studies that have shown that cancerous tissues often exhibit distinct microbial communities compared to non-cancerous tissues. The presence of specific microbial species in cancer patients might reflect changes in the local microenvironment caused by tumor growth or the body's response to malignancy.

Specific Microbial Species Associated with Bladder Cancer

Our study identified several bacterial species that were differentially abundant between the BC and non-BC groups. Notably, species such as *Bacteroides fragilis*, *Escherichia coli*, and *Fusobacterium nucleatum* were significantly enriched in the urine of bladder cancer patients. These bacteria are known to be associated with inflammation and immune modulation, both of which are crucial factors in cancer development and progression. *Bacteroides fragilis* has been implicated in various cancers due to its ability to produce toxins that can induce DNA damage and promote inflammation. Similarly, *Escherichia coli* is a well-known pathogen that has been linked to bladder cancer through chronic infection and the production of carcinogenic metabolites such as nitrosamines. The presence of these bacteria in the urine of bladder cancer patients suggests that they may play a role in the initiation or progression of bladder cancer, possibly by promoting local inflammation or by altering the local immune response. *Fusobacterium nucleatum*, another bacterium found to be more abundant in BC patients, is known for its role in promoting inflammation and immune evasion. Studies have shown that *F. nucleatum* can promote the development of colorectal cancer by influencing the tumor microenvironment and modulating immune responses. While its role in bladder cancer is not yet well understood, its increased abundance in our BC group suggests it may also be involved in bladder carcinogenesis. The finding of these specific microbial taxa reinforces the hypothesis that microbial dysbiosis, especially an overrepresentation of bacteria associated with inflammation, could contribute to the development of bladder cancer. These bacteria may create a tumor-friendly environment by promoting immune evasion, enhancing local inflammation, or disrupting the normal function of the urinary tract.

Urinary Metabolomics and Its Role in Bladder Cancer Detection

In addition to microbial profiling, we conducted a metabolomics analysis to assess the urinary metabolic changes associated with bladder cancer. Our results showed that several metabolites, including short-chain fatty acids (SCFAs) and bile acids, were significantly more abundant in the urine of BC patients. SCFAs such as acetate, propionate, and butyrate, which are primarily produced by gut microbiota, were notably elevated in the BC group. These metabolites are often considered beneficial in maintaining gut health, as they have anti-

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inflammatory and immune-regulatory properties. However, their increased levels in the urine of bladder cancer patients may indicate a dysregulated metabolic process linked to cancer. Inflammation is a hallmark of cancer, and SCFAs play a critical role in regulating inflammation by modulating immune cell activity. The overproduction of SCFAs in our study could reflect an altered immune response, possibly contributing to the chronic inflammation observed in bladder cancer. Bile acids, which are involved in lipid digestion and absorption, also showed significant differences between the BC and non-BC groups. Taurocholic acid and cholic acid, two bile acids that were found to be more abundant in the BC group, have been implicated in various forms of cancer, including colon and liver cancer. Bile acids are known to affect cell signaling and inflammation, and their altered levels in cancer patients may be a consequence of both tumor growth and microbial dysbiosis. The increased concentration of these metabolites in bladder cancer patients suggests that bile acid metabolism might be disrupted in the context of bladder cancer, providing further evidence for the involvement of microbial metabolites in carcinogenesis. Interestingly, we also observed elevated levels of certain amino acids, such as glutamine, glycine, and leucine, in the urine of bladder cancer patients. Amino acids play a crucial role in cellular metabolism, and their altered levels in cancer patients are well-documented. Glutamine, for example, is a key metabolic intermediate in many cancer cells and is essential for supporting the increased energy demands of rapidly proliferating tumor cells. The elevated levels of glutamine in the BC group may reflect the altered metabolic state of cancer cells, which often rely on amino acid metabolism to fuel growth.

The Correlation Between Microbes and Metabolites

A key finding of our study was the correlation between specific microbial taxa and metabolites in the urine of bladder cancer patients. We observed that bacteria such as *Bacteroides fragilis* and *Escherichia coli* were strongly correlated with metabolites like butyrate and cholic acid. This correlation suggests that the microbial community in the urine may directly influence metabolic pathways involved in bladder cancer progression. The presence of specific bacterial species could lead to the production of metabolites that enhance inflammation or promote tumor growth. For example, *E. coli* is known to produce nitrosamines, which are carcinogenic compounds that could directly contribute to the development of bladder cancer.

Furthermore, the significant correlations between SCFAs and specific bacterial species highlight the importance of the microbiome in modulating metabolic processes. SCFAs produced by certain bacteria might not only serve as energy sources for host cells but could also influence immune responses and inflammation. The microbiome's role in regulating these metabolic pathways is increasingly recognized as a critical factor in cancer development, and our findings provide further support for this concept.

Potential of Urinary Microbial Metabolites as Biomarkers for Early Detection of Bladder Cancer

The ultimate goal of this study was to assess the potential of urinary microbial metabolites as non-invasive biomarkers for the early detection of bladder cancer in diabetic patients. Based on our results, we conclude that certain microbial metabolites, particularly SCFAs and bile acids, could serve as valuable biomarkers for early bladder cancer detection. These metabolites are easily detectable in urine and have the potential to be used in routine clinical practice for non-invasive screening of bladder cancer. The advantage of using urine for biomarker detection lies in its accessibility and the non-invasive nature of sample collection, which makes it an ideal tool for early cancer diagnosis.

While the results of this study are promising, further research is needed to validate the identified biomarkers in larger, independent cohorts. The relationship between microbial metabolites and bladder cancer is complex and multifactorial, and additional studies should aim to clarify the specific mechanisms through which microbial dysbiosis contributes to cancer progression. Moreover, it will be important to investigate whether these biomarkers can be used to detect bladder cancer at earlier stages, before it becomes clinically apparent.

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Limitations and Future Directions

One limitation of this study is the relatively small sample size, which may limit the generalizability of the findings. While we observed significant differences between the BC and non-BC groups, larger studies with more diverse populations are needed to confirm the results and determine the robustness of the identified biomarkers. Additionally, the cross-sectional nature of this study does not allow us to establish causality between microbial metabolites and bladder cancer. Future studies with longitudinal designs will be crucial to determine whether microbial metabolites precede the development of bladder cancer or if they are a consequence of tumor progression.

Another area for future research is the exploration of the specific mechanisms through which the identified microbial species and metabolites contribute to bladder cancer. Understanding these mechanisms could lead to the development of targeted interventions aimed at modulating the microbiome to prevent or treat bladder cancer. Additionally, integrating microbial metabolite profiles with genetic and clinical data could enhance the accuracy of early cancer detection and improve patient outcomes.

CONCLUSION

This study provides compelling evidence that urinary microbial metabolites could serve as valuable, non-invasive biomarkers for the early detection of bladder cancer (BC) in diabetic patients. Our findings demonstrate significant differences in microbial diversity and metabolic profiles between diabetic patients with and without bladder cancer, suggesting that microbial dysbiosis and altered metabolic processes are key features of bladder cancer development in this high-risk group. Specific bacterial species, such as *Bacteroides fragilis* and *Escherichia coli*, were found to be enriched in the urine of BC patients, alongside elevated levels of metabolites like short-chain fatty acids and bile acids. The observed correlation between microbial taxa and metabolites further supports the idea that the urinary microbiome plays a crucial role in shaping the metabolic landscape in bladder cancer. These metabolites, which are easily detectable in urine, have the potential to be utilized as non-invasive biomarkers that could simplify and enhance the early diagnosis of bladder cancer, especially in diabetic patients who are at higher risk. This approach could reduce the need for more invasive and costly diagnostic procedures, such as cystoscopy, and provide a simpler method for identifying bladder cancer at earlier, more treatable stages. While the results of this study are promising, they are preliminary, and further validation through larger, multicenter studies is necessary to confirm the robustness and clinical applicability of these biomarkers. Future research should focus on longitudinal studies to understand the temporal relationship between microbial dysbiosis, metabolic changes, and bladder cancer development, as well as investigating the potential therapeutic implications of modulating the microbiome in preventing or treating bladder cancer.

In conclusion, urinary microbial metabolites hold great promise as a diagnostic tool for early bladder cancer detection in diabetic patients. By continuing to explore the intricate relationship between the microbiome, metabolism, and cancer, we may be able to develop more effective, non-invasive screening methods that could improve outcomes and reduce the burden of bladder cancer on patients and healthcare systems.

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