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The biosensor using the target urine volatile organic compounds for detecting diabetic kidney disease

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Volatile organic compounds (VOCs) are metabolic byproducts reflecting pathophysiologic processes and have been widely utilized as non-invasive biomarkers for disease differentiation. While VOCs have been extensively studied in oncology, their potential for diagnosing other prevalent public health conditions remains underexplored. This study serves as a proof-of-concept investigation, applying VOC-based detection to diabetic kidney disease (DKD), a leading cause of chronic kidney disease, to distinguish it from other nephrotic syndromes (NS). Utilizing metal oxide semiconductors, we analyzed urinary VOC profiles in 135 participants with biopsy-confirmed diagnoses. All gas sensors in the sensor chamber were of the n-type. Our results demonstrated that hydrogen and ethanol VOCs, measured at a minimum electrical resistance threshold of 4500 mV, effectively distinguished DKD from NS and other control groups. The mean differences in electrical resistance among DKD versus normal, DM without DKD, and NS were 868.3, 145.5, and 881.2 ohms, respectively ($p < 0.05$). Receiver operating characteristic (ROC) analysis yielded AUC values of 1.0, 0.64, and 0.99, respectively ($p < 0.05$), underscoring the method's diagnostic potential. VOCs primarily composed of hydrogen and ethanol in DKD patients exhibited distinct electron-release properties when interacting with metal oxide semiconductors compared to healthy individuals and NS patients. These findings suggest that urine-based VOC analysis has potential as a non-invasive alternative for distinguishing DKD from other glomerular diseases. This study highlights the feasibility of extending VOC-based biosensor technology beyond oncology, offering a novel diagnostic framework for broader clinical applications in public health and metabolic disease monitoring.

Keywords Diabetic kidney disease (DKD), Diabetic nephropathy (DN), Volatile organic compounds (VOCs), Biosensor

Diabetic kidney disease (DKD) is a common complication, affecting approximately 20–40% of individuals with diabetes mellitus. It remains the leading cause of chronic kidney disease. However, its detection is often delayed, particularly in type 2 diabetes, due to prolonged periods of asymptomatic hyperglycemia. Over time, chronic kidney disease progresses to kidney failure, with diabetic retinopathy frequently coexisting with DKD[1]. Patients diagnosed with diabetes-related retinopathy and concurrent kidney disease are often presumed to have DKD as the primary cause of kidney impairment. However, other primary glomerular diseases may also contribute to renal dysfunction. Therefore, a kidney biopsy is required to confirm the underlying cause and guide appropriate treatment. Nevertheless, as an invasive procedure, kidney biopsy carries risks, including abdominal wall bleeding, potential kidney failure, and, in rare cases, death. As a result, some patients refuse the procedure, while physicians may overlook its necessity, assuming DKD as the sole cause of kidney failure, particularly in individuals without diabetic retinopathy[2,3]. Despite ongoing research and the development of novel diagnostic methods, such as

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the utilization of the rs1888747 polymorphism in the FRMD3 gene, challenges persist in accurately diagnosing DKD. Furthermore, this approach has yet to gain widespread acceptance in clinical practice[4,5].

Currently, a growing number of studies focus on leveraging distinct phenotypic features to identify pathophysiological characteristics[6,7]. Volatile organic compounds (VOCs) have gained increasing attention for cancer screening and diagnosis, with successful applications in the preliminary detection of lung, breast, colon, and other cancers[8,9]. These compounds are naturally produced through environmental processes and as metabolic by-products in the human body. They can be detected in breath, sweat, saliva, feces, and urine. Moreover, VOC detection is less complex than other molecular biology methods, as it does not require extensive sample preparation or advanced laboratory infrastructure. The use of commercial gas sensors allows real-time analysis, making this approach both practical and cost-effective for diagnostic applications.

Several studies have highlighted the correlation between urinary VOCs and kidney diseases, identifying compounds such as carbamic acid mono ammonium salt, 2-pentanone, 2,4-dimethyl-pentanal, hydrogen azide, thiourea, and 4-heptanone in association with primary glomerular diseases. Likewise, specific VOCs have been linked to diabetes-related diseases, including 2-aminothiazoline-4-carboxylic acid, N-acetyl-S-(2-carboxyethyl)-L-cysteine, phenyl glyoxylic acid, and N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine. However, confirmatory VOC detection methods, such as gas chromatography or ultraperformance liquid chromatography coupled with electrospray ionization tandem mass spectrometry, remain complex and expensive, limiting their feasibility for routine clinical use[10–15].

Our previous work demonstrated that VOC biosensors could effectively detect genitourinary cancers by analyzing changes in electrical resistance induced by specific urinary VOCs. That study identified methane, isobutane, hydrogen, and ethanol as significant biomarkers, highlighting the potential of VOC-based diagnostics in oncology[16]. These findings align with other research recognizing VOCs as promising biomarkers for cancer detection, reinforcing their role in non-invasive diagnostic strategies[17,18]. Building on these findings, this study extends the application of VOC biosensors beyond cancer detection to address a broader public health issue—DKD. Rather than identifying specific VOCs, we investigate whether VOC-induced electrical resistance changes can distinguish DKD from other nephrotic syndromes (NS). This approach shifts the focus from cancer screening to metabolic and renal disease differentiation, emphasizing VOCs as a versatile diagnostic tool. If resistance patterns in DKD prove sufficiently distinct, this method could provide a non-invasive alternative to kidney biopsy, addressing a critical gap in nephrology diagnostics.

Materials and methods

Clinical data collection

This study was conducted as a descriptive cohort study. General patient data, including age, gender, medications, and chronic diseases that could potentially affect VOC test results, were reviewed and recorded from medical records of individuals attending the outpatient clinic at Suranaree University of Technology Hospital between March 2024 and July 2024. The protocol for VOC detection in urinary cancer, developed in our previous research[16], was applied. Given the limited application of VOC analysis in diagnosing diseases beyond cancer, this study aimed to serve as a proof of concept to demonstrate the feasibility of applying VOC protocols to non-cancerous conditions, such as DKD and NS. All experimental protocols, including the use of biosensor technology and associated methodologies, were thoroughly reviewed and approved by the Human Research Ethics Board of Suranaree University of Technology (Approval Issue # EC-67–0001). The study was conducted in strict compliance with relevant regulations and guidelines.

All patients were required to be over 20 years old and capable of providing informed consent independently. Female patients underwent additional pregnancy testing, for which separate informed consent was obtained. Pregnant individuals were excluded. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI 2021 equation, as documented in the patients' medical records[19].

The study population was selected based on pathology-matched diseases. The adequacy of the study population for comparing DKD to other groups was calculated using an infinite population proportion with a 10% margin of error and a 95% confidence interval Z-score. The required sample size was determined using the Sample Size Formula for Population Proportion: $n = (Z^2 * p * (1 - p))/ E^2$, where n was the required sample size, $Z = 1.96$ (95% confidence level), $p = 0.5$ (conservative estimate), and $E = 0.1$ (10% margin of error). To ensure a balanced comparison between groups (DM with DKD, DM without DKD, and NS), the minimum required sample size was approximately 110 participants, which was deemed sufficient for analysis.

Renal biopsies were performed within one month following the detection of clinically significant proteinuria (> 1 g/day), classified as glomerular-range proteinuria. This proteinuria level was typically indicative of glomerular diseases and warranted confirmation through renal biopsy. Conversely, proteinuria levels below 1 g/day were often attributed to extraglomerular causes, making a biopsy unnecessary. The study followed a descriptive diagnostic framework using new technological methods without interventions between study groups.

Urine collection for target VOCs

Each participant in the study was asked to provide a 20 mL urine sample. The collected samples were analyzed using a target urine VOC biosensor, as described in the study available at this link https://www.nature.com/articles/s41598-024-54138-1#Sec2 [16].

The VOC analysis involved capturing the evaporated VOCs from the urine container as they diffused into a sealed chamber. These VOCs were then simultaneously analyzed using five gas semiconductor sensors positioned at the top of the chamber. **(Supplementary Fig. 1)**.

Clinical and laboratory evaluation

Proteinuria and albuminuria levels were assessed using 24-hour urine protein, 24-hour urine albumin, the urine protein-creatinine ratio, and the urine albumin-creatinine ratio. These measurements were analyzed using benzethonium chloride and enzymatic methods with the ARCHITECT C8000 analyzer (Abbott, Abbott Park, IL, USA). The eGFR was calculated based on serum creatinine and clinical data using the CKD-EPI Creatinine Eq. (2021)[19,20].

Gas sensors

Five distinct commercially available metal oxide semiconductor sensors manufactured by Figaro Engineering Inc. were utilized, selected based on evidence from previous clinical studies[16].

Gas sensor analysis

All gas sensors positioned within the sensor chamber were composed of n-type tin (Sn) oxide. The VOCs resulting from cellular metabolism acted as reducing gases. When the targeted VOCs evaporated into the conducting chamber, they reacted with oxygen ions in the gas sensors, releasing free electrons. This electron release led to a decrease in electrical resistance, increasing sensor conductivity. In simpler terms, as VOCs interacted with the sensors, electrical resistance decreased, enhancing conductivity.

The five gas sensors were selected based on their prior validation as commercial VOC biosensors in cancer studies. This study aimed to explore whether these sensors could detect VOCs associated with non-cancerous diseases, such as DKD and NS. The rationale behind this selection was to evaluate the capability of these sensors in detecting VOCs, for which data in non-cancerous conditions remain limited.

Additionally, variations in temperature within the conducting chamber influenced metal oxide decomposition and the subsequent release of free electrons, with effects specific to each temperature level **(Supplementary Fig. 2)**. The conducting chamber was designed using commercially available metal oxide sensors manufactured by Figaro Engineering Inc. The heater's operating voltage was set to generate the highest degree of heat, approximately 5000 ± 200 mV DC, as recommended by the manufacturer. The electrical voltage was adjusted to produce five different heat energy levels (2000 mV, 2500 mV, 3500 mV, 4500 mV, and 5000 mV), each applied for 80 s. The protocol involved administering electrical current to the heater chambers while monitoring electrical resistance changes in each sensor. Detailed protocols are available in previous research[16].

Clinical allocation and assessment

Urine samples were categorized into four groups for statistical analysis: (1) normal subjects, (2) individuals with diabetes exhibiting low albuminuria (< 30 mg/g creatinine) and presumed to be without DKD (DM without DKD), (3) those diagnosed with DKD, and (4) individuals with other glomerular diseases, such as lupus nephritis and primary glomerulonephritis (NS). Patients with eGFR < 15 mL/min/1.73 m^2 were excluded. Additionally, participants in the normal subjects and diabetes with low albumin groups, despite not undergoing kidney biopsy, were required to have normal urine analysis results documented at least twice consecutively with a four-week interval. Their 24-hour urine albumin or urine albumin-creatinine ratio had to be less than 30 mg/day or 30 mg/g creatinine, respectively, and documented at least twice consecutively with a four-week interval. Patients with urine albumin levels between 30 and 300 mg/day or mg/g creatinine, as measured by the 24-hour urine albumin or urine albumin-creatinine ratio method[21], were considered to have semi-positive results and were excluded from the study. However, patients diagnosed with DKD, or other glomerular diseases (nephrotic syndrome) were required to have pathology-confirmed results from kidney biopsy for inclusion.

Patients whose pathology results did not indicate a specific disease, had semi-positive pathology findings, were diagnosed with more than two types of kidney diseases, or had extraglomerular conditions were excluded from the study. To ensure diagnostic accuracy, all pathology results were reviewed by two independent pathologists. If their evaluations differed or the results were inconclusive, the patient was excluded. This rigorous selection process was implemented to minimize misclassification and enhance the reliability of the findings.

Patients diagnosed with DKD were required to have Class II pathological results based on the Pathologic Classification of Diabetic Nephropathy (2010)[22]. This classification defines Class II as diffuse diabetic glomerulosclerosis with extracellular material expansion in the mesangium, where the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules[22].

The comparison of VOCs through changes in electrical resistance in this study followed a protocol previously reported for comparing urine VOCs of patients with genitourinary cancer to those who were cancer-free[16]. Changes in electrical resistance from the baseline to the bottom line were observed and compared across five semiconductors. Semiconductor No.1 (S1) targeted VOCs of methane and iso-butane, Semiconductor No.2 (S2) targeted VOCs of hydrogen and ethanol, Semiconductor No.3 (S3) targeted VOCs of hydrogen, sulfide, and ammonia, Semiconductor No.4 (S4) targeted VOCs of toluene, butane, and propane, and Semiconductor No.5 (S5) targeted VOCs of trimethylamine and methyl-mercaptan. All semiconductors were manufactured by Figaro Engineering Inc. (https://www.figarosensor.com/product/sensor/).

Electrical resistance variables

Due to the disease-specific and distinct characteristics of VOCs, as well as variations in emission patterns caused by changes in temperature and VOC concentrations unique to each disease, this study conducted VOC testing in urine by applying heat six times. The electrical voltage was adjusted to produce five different levels of heat energy, starting at 2000 mV and increasing by 500–1000 mV every 80 s. This process was repeated six times until reaching the maximum electrical voltage of 5000 mV, following the protocol from a previous study[16]. The summarization and simplification of biosensor variables, including the minimum and time values of each electrical voltage release repeated six times across five biosensors, are presented in **Supplementary Fig. 3.**

Statistical analysis

Data analysis was conducted using R software version 2.9.1 and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were expressed as percentages. Differences between groups were compared using Student's t-test for continuous variables and Pearson's chi-square test for categorical variables. The Spearman correlation coefficient was applied for selection algorithm assessments. Diagnostic accuracy was evaluated using receiver operating characteristic (ROC) analysis, with p-values ≤ 0.05 considered statistically significant.

Results

Participants' characteristics

The authors evaluated urinary VOCs in a cohort of 135 participants **(Supplementary Table 1)**. This cohort included 9 (6.7%) healthy volunteers with normal results in renal function tests, urine examinations, and fasting blood sugar tests. Additionally, 52 (38.5%) participants were diabetic patients with a urine albumin-creatinine ratio < 30 mg/g creatinine, presumed to be DM without DKD. There were also 50 (37%) diabetic patients with confirmed pathology results indicating DKD, and 24 (17.8%) patients with nephrotic syndrome from various causes, including lupus nephritis (25%), primary membranous nephropathy (25%), IgA nephropathy (12.5%), minimal change disease (12.5%), and FSGS (25%). None of these nephrotic syndrome patients had concurrent diabetes, and all were diagnosed through tissue pathology. All diabetic patients in this study had type 2 DM.

The patients who participated in the study received treatment from their attending internists. The researchers did not intervene in the treatment involving oral hypoglycemic drugs, insulin, or any other medications affecting blood sugar levels and proteinuria. According to the data in **Supplementary Table 1**, the age, fasting blood sugar (FBS) levels, and HbA1C levels of diabetic patients were higher than those of patients in other groups. However, there were no statistically significant differences between diabetic patients with and without DKD, nor between normal subjects and the group with other causes of nephrotic syndrome.

The level of proteinuria was significantly higher in the group with other causes of nephrotic syndrome compared to all other groups, including the DM with DKD group ($p < 0.001$). Additionally, although proteinuria levels in the DM without DKD group were lower than those in the DM with DKD and other nephrotic syndrome groups, they were still significantly higher than those in the normal subjects ($p < 0.001$).

Urine VOC characteristics in patients with DM and DKD

When comparing electrical resistance variables of each semiconductor across urine samples from patients in each group using Analysis of Variance (ANOVA), it was observed that there were significant differences in the changes in almost every variable of each group across all semiconductors. However, in semiconductors S1, S4, and S5, no differences in electrical resistance variables were detected between disease groups when the electrical current exceeded 2500 mV. In contrast, semiconductors S2 and S3 showed differences in urine VOCs across every heat energy interval. Consequently, the researchers chose to present the specific mean and standard deviation data of electrical resistance variables from biosensors S2 and S3 in Supplementary Table 2, while the data from all semiconductors were included in the **Supplementary Analysis**.

In semiconductor S2, VOCs from normal individuals entering the conducting chamber yielded the lowest electrical resistance values (min1, mean = 7939.1 ± 7.8 ohms) compared to patients with other NS (mean = 7944.4 ± 10.4 ohms) and diabetic patients both with DKD (mean = 7985.0 ± 11.1 ohms) and without DKD (mean = 7988.5 ± 2.5 ohms), respectively ($p < 0.001$).

Upon releasing heat energy, it was observed that VOCs from patients in the other NS group had a minimum electrical resistance value at the end of the experiment (min6) that was lower than that of normal individuals ($p < 0.001$). This finding indicated that VOCs from the pathophysiology in the other NS group had a greater capability to adhere to metal oxide and release free electrons compared to VOCs from normal individuals.

Interestingly, while the diabetic group yielded higher min6 values than normal individuals and patients in the other NS group, the min1 value of DM with DKD (mean = 7985.0 ± 11.1 ohms) was lower than that of DM without DKD (mean = 7988.5 ± 2.5 ohms) ($p < 0.001$). Conversely, compared to normal individuals and patients in the other NS group, the VOCs of DM with DKD exhibited higher min6 values (mean = 6329.3 ± 121.6 ohms) than those of the DM without DKD group (mean = 6292.5 ± 149.6 ohms) ($p < 0.001$). This finding suggested that VOCs from the pathophysiology in the DKD group had a reduced capability to adhere to metal oxide and release free electrons compared to VOCs associated with prior diabetic pathophysiology.

When considering the time to adhere to metal oxide (time), it was found that normal individuals had the fastest time2 value. The time2 value for NS and DM without and with DKD was approximately 2.5 and 5 times slower, respectively ($p < 0.001$). Upon releasing heat energy until the end of the experiment, time6 for each group's VOCs adhering to metal oxide accelerated but still took longer than in normal individuals ($p < 0.001$).

In contrast, **semiconductor S3** revealed that the lowest min1 and min6 values in each patient group followed a pattern similar to those in semiconductor S2. Regarding the speed of adherence to metal oxide, when heat energy was released until the end of the experiment, the time6 value for VOCs from NS patients demonstrated faster adherence to metal oxide and released free electrons more quickly compared to VOCs from normal individuals. Similarly, diabetic patients exhibited time6 values that aligned with the trends observed in S2 when compared to normal individuals.

Urine VOCs difference among diabetic patients with or without DKD and other nephrotic syndrome

To discern pairwise differences in comparing DKD with other NS—an essential consideration in clinical practice for deciding whether a renal biopsy should be performed to identify coexisting causes of NS in diabetic patients—data from S2 and S3 were utilized (data from other semiconductors were provided in the Supplementary

Analysis). Mean differences between min and time values for each interval of time when electrical current was released were calculated using an independent t-test.

Specific response patterns of VOCs in each group were identified according to the different levels of heat energy in the conducting channel. These patterns were disease-specific and varied among different types of semiconductors in every interval where heat energy > 2000 mV was applied (time2–time5) for both S2 and S3, as shown in Table 1. VOCs from DM patients with DKD were compared against other groups using an independent t-test for each pair, as indicated in Table 1. Additional data was presented in the **Supplementary Analysis**.

From the comparison of the mean differences in the minimum electrical resistance points (min) of semiconductors S2 and S3 between the study groups, DM with DKD exhibited distinctly different VOC characteristics compared to the Normal group, DM without DKD, and other causes of NS during cycles 1–4 (min1–min4) when electrical current was released. However, when comparing the mean differences using the time variable, VOCs from DM with DKD could not be distinguished from those without DKD. Additionally, the direction of the time interval for releasing and capturing metal oxide of VOCs in each group occurred inconsistently.

The data indicated that electrical resistance variables differed between disease groups, allowing for differentiation. However, the min variables of both S2 and S3, particularly min1–min4, showed inconsistent patterns in time variables. As a result, ROC analysis was conducted to assess the ability to differentiate DKD from other diseases, as shown in Table 2 (data from other semiconductors are provided in the **Supplementary Analysis**).

Analysis revealed that the ability to differentiate DM with DKD from other disease groups, including Normal, DM without DKD, and other causes of NS, was present only in S2 during the min1–min4 intervals. The AUC of the ROC was greater than 0.5, indicating significant diagnostic capability. Specifically, S2min4 demonstrated the highest AUC of the ROC for distinguishing DM with DKD from other diseases, with AUC values of 1.000,

	DM with DKD vs. Normal		DM with DKD vs. without DKD		DM with DKD vs. other causes of NS	
	Mean difference (95%CI)	*p*-value	Mean difference (95%CI)	*p*-value	Mean difference (95%CI)	*p*-value
Min						
Semiconductor No. 2						
S2 min1	45.85 (38.11, 53.59)	< 0.001	− 3.5 (− 6.7, − 0.3)	0.032	40.6 (32.2, 48.97)	< 0.001
S2 min2	1851.1 (1618.6,2083.6)	< 0.001	129.6 (26.6, 232.6)	0.014	1428.6 (848.9, 2008.4)	0.001
S2 min3	1806.7 (1613.7,1999.6)	< 0.001	201.6 (52.8, 350.5)	0.009	1608.3(1195.6, 2021.1)	< 0.001
S2 min4	868.3 (710.4, 1026.2)	< 0.001	145.5(33.5, 257.6)	0.011	881.2 (707.9, 1054.4)	< 0.001
S2 min5	311.3 (214.5, 408.1)	< 0.001	48.5 (− 9.1, 106.1)	**0.098**	405.3 (299.2, 511.4)	< 0.001
S2 min6	225.5 (89.7, 361.2)	0.004	36.9 (− 16.8, 90.5)	**0.176**	390.0 (204.1, 575.8)	0.001
Semiconductor No. 3						
S3 min1	64.4 (8.5, 120.2)	0.029	− 3.9 (− 7.1, -0.7)	0.019	64.6 (32.8, 96.3)	0.002
S3 min2	560.7 (469.9, 651.4)	< 0.001	− 8.6 (− 15.9, -1.4)	0.021	547.9 (457.3, 638.6)	< 0.001
S3 min3	946.0 (765.8, 1126.3)	< 0.001	− 13.4 (− 25.8, -1.1)	0.034	1020.7 (917.1, 1124.4)	< 0.001
S3 min4	784.4 (592.1, 976.8)	< 0.001	− 14.2 (− 28.1, -0.2)	0.047	1026.5 (934.1, 1118.9)	< 0.001
S3 min5	445.4 (286.7, 604.2)	< 0.001	− 7.7 (− 18.1, 2.7)	**0.145**	706.4 (623.2, 789.6)	< 0.001
S3 min6	267.9 (132.0, 403.7)	0.002	− 2.5 (− 10.9, 5.9)	**0.556**	512.3(423.4, 601.2)	< 0.001
Time						
Semiconductor No. 2						
S2 time1	327.2 (297.8, 356.6)	< 0.001	10.6 (− 12.1, 33.3)	**0.356**	235.5 (110.1, 360.8)	0.003
S2 time2	344.5 (288.2, 400.8)	< 0.001	78.9 (29.2, 128.6)	0.002	338.9 (279.1, 398.8)	< 0.001
S2 time3	13.3 (11.8, 14.9)	< 0.001	2.3 (− 0.4, 4.9)	**0.089**	12.0 (10.3, 13.7)	< 0.001
S2 time4	2.5 (1.4, 3.6)	< 0.001	0.5 (− 0.2, 1.2)	**0.14**	1.6 (0.4, 2.8)	0.008
S2 time5	1.8 (0.7, 2.8)	0.001	0.5 (− 0.1, 1.1)	**0.096**	− 0.2 (− 1.5, 1.1)	**0.805**
Semiconductor No. 3						
S3 time1	44.3 (− 57.9, 146.5)	**0.347**	–	N/A	49.9 (− 68.1, 167.8)	**0.351**
S3 time2	249.9 (203.1, 296.7)	< 0.001	–	N/A	154.3 (61.5, 247.0)	0.006
S3 time3	5.5 (1.2, 9.7)	0.013	1.0 (− 1.5, 3.5)	**0.411**	− 5.3 (− 9.8, − 0.9)	0.02
S3 time4	14.8 (12.6, 17.0)	< 0.001	− 3.6 (− 6.5, -0.7)	0.016	11.0 (8.6, 13.3)	< 0.001
S3 time5	307.9 (271.8, 344.0)	< 0.001	− 48.5 (− 86.5, − 10.5)	0.013	305.6 (269.5, 341.7)	< 0.001
S3 time6	306.9 (219.2, 394.6)	< 0.001	2.5 (− 3.1, 8.2)	**0.377**	339.1 (334.8, 343.5)	< 0.001

Table 1. Mean difference of the electrical variables between groups. N = number, DM without DKD = diabetes without diabetic kidney disease, DM with DKD = diabetes with diabetic kidney disease, NS = nephrotic syndrome, N/A = not applicable, sec = seconds, SD = standard deviation, bold number = not significant, CI = confidence interval.

Test Result Variable(s)	DM with DKD vs. Normal				DM with DKD vs. DM without DKD				DM with DKD vs. other NS			
	AUC	95% Confidence Interval		*p*-value	AUC	95% Confidence Interval		*p*-value	AUC	95% Confidence Interval		*p*-value
Semiconductor No.2												
S2 min1	1.000	1.000	1.000	< **0.001**	0.526	0.408	0.645	0.649	1.000	1.000	1.000	< **0.001**
S2 min2	1.000	1.000	1.000	< **0.001**	0.626	0.513	0.740	**0.028**	0.993	0.975	1.000	< **0.001**
S2 min3	1.000	1.000	1.000	< **0.001**	0.640	0.530	0.750	**0.015**	0.993	0.975	1.000	< **0.001**
S2 min4	*1.000*	*1.000*	*1.000*	< ***0.001***	*0.642*	*0.533*	*0.752*	***0.013***	*0.995*	*0.982*	*1.000*	< ***0.001***
Semiconductor No.3												
S3 min1	1.000	1.000	1.000	< **0.001**	0.483	0.364	0.602	0.766	1.000	1.000	1.000	< **0.001**
S3 min2	1.000	1.000	1.000	< **0.001**	0.529	0.413	0.645	0.613	1.000	1.000	1.000	< **0.001**
S3 min3	1.000	1.000	1.000	< **0.001**	0.528	0.411	0.644	0.630	1.000	1.000	1.000	< **0.001**
S3 min4	1.000	1.000	1.000	< **0.001**	0.517	0.402	0.632	0.771	1.000	1.000	1.000	< **0.001**

Table 2. Area under the ROC curve of minimal electrical resistance of VOCs predicting DKD. ROC = receiver operating characteristic, DM without DKD = diabetes without diabetic kidney disease, DM with DKD = diabetes with diabetic kidney disease, NS = nephrotic syndrome, bold number = statistically significant.

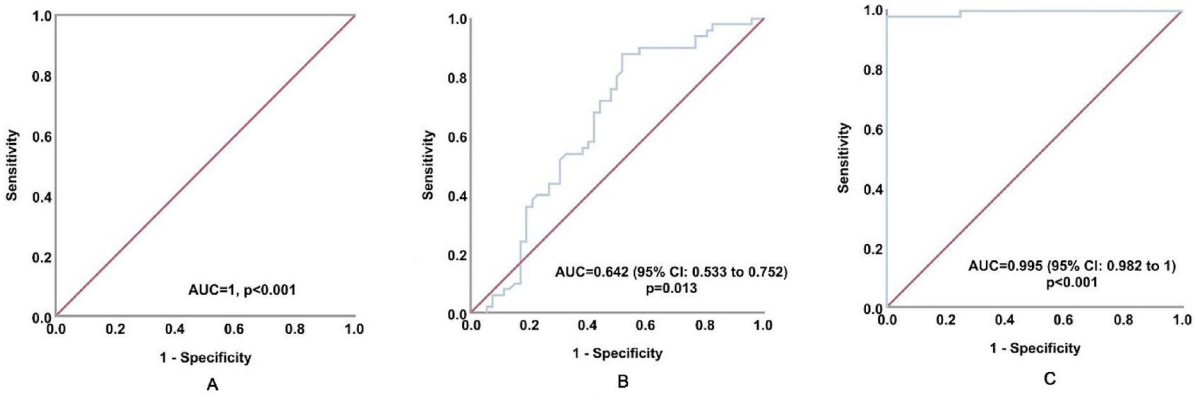

Fig. 1. The Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC); **A** showed the ability to distinguish DM with DKD from Normal, **B** showed the ability to distinguish DM with DKD from DM without DKD, and **C** showed the ability to distinguish DM with DKD from other causes of NS. In all three cases, the AUCs were 1.00, 0.642, and 1.00, respectively, with p-value < 0.05. DM without DKD = diabetes without diabetic kidney disease, DM with DKD = diabetes with diabetic kidney disease, NS = nephrotic syndrome, AUC = area under the receiver operating characteristic curve, CI = confidence interval.

0.642, and 0.995 for differentiating from the Normal group, DM without DKD, and other NS, respectively—all of which were clinically significant ($p < 0.05$).

To reinforce the reliability of these findings, we conducted a power analysis using the Power for Comparing Sensitivities from Two Independent Populations formula. Given that this study aims to establish the specificity of VOCs in differentiating DKD from DM without DKD and other glomerular diseases, the power calculation was performed on the subgroup with the smallest sample size and the lowest sensitivity value (54%). This approach ensured that the statistical inference remained valid even under the most challenging conditions. The power analysis yielded a value of 0.989 (> 0.8), confirming a strong predictive ability and supporting the robustness of the study design[23]. Unlike Effect Size calculations, which often rely on assumptions of Normal Distribution, the Power of Test method focuses on detecting true outcomes, making it a more suitable statistical tool for clinical research where data distributions may vary[23–25]. The high-power value obtained further substantiates that, even under conditions with the lowest sensitivity, VOC-based detection of DKD remains both practically useful and statistically reliable.

Building on these findings, S2min4 emerged as a key semiconductor variable for distinguishing DM with DKD from other conditions. The ROC curve was plotted to evaluate the sensitivity and specificity for diagnosing DKD (Fig. 1 **and** Table 3). Further analysis indicated that the characteristic VOC pattern in patients with DM with DKD, when examined using semiconductor S2 with an electrical current of 4500 mV, produced a specific minimum electrical resistance level (S2min4) that clearly distinguished this group from Normal, DM without DKD, and other NS. The cut-off electrical resistance values for distinguishing DM with DKD were greater than 5536, 6285, and 5736 ohms, respectively ($p < 0.05$). However, when focusing on the distinction between DM with

Clinical determination	Diagnostic testing accuracy						
	AUC	Electrical resistance cut off (ohm)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
DM with DKD vs. Normal	1.00	5536	100	100	100	100	100
DM with DKD vs. DM without DKD	0.642	6285	54.0	67.3	61.4	60.3	60.8
DM with DKD vs. Other causes of NS	0.995	5736	98.0	100	100	88.9	98.3

Table 3. Diagnostic testing accuracy in the diagnosis between DM with DKD and other conditions. DM without DKD = diabetes without diabetic kidney disease, DM with DKD = diabetes with diabetic kidney disease, NS = nephrotic syndrome, AUC = area under the receiver operating characteristic curve, PPV = positive predictive value, NPV = negative predictive value.

and without DKD, the diagnostic accuracy was reduced, though it remained statistically significant (Fig. 1B), suggesting shared pathophysiological characteristics between the two diabetic populations.

Further analysis indicated that the characteristic VOC pattern in patients with DM with DKD, when examined using semiconductor S2 with an electrical current of 4500 mV, produced a specific minimum electrical resistance level (S2min4) that clearly distinguished this group from Normal, DM without DKD, and other NS. The cut-off electrical resistance values for distinguishing DM with DKD were greater than 5536, 6285, and 5736 ohms, respectively ($p < 0.05$). However, when focusing on the distinction between DM with and without DKD, the diagnostic accuracy was reduced, though it remained statistically significant (Fig. 1B), suggesting shared pathophysiological characteristics between the two diabetic populations.

If the minimum electrical resistance levels for separating DM with and without DKD were used as a shared reference point for diagnosing DM with DKD from other conditions, patients exhibiting a characteristic VOC pattern examined with semiconductor S2 and resistance levels above 6285 ohms could be differentiated from Normal individuals with a specificity and accuracy of 100% and 59.3%, respectively, and from other NS causes with a specificity and accuracy of 100% and 58.6%, respectively. This approach allowed for the diagnosis of DM with DKD from other conditions without the need for a renal biopsy.

Discussion

DKD is widely recognized as a major complication that can progress to end-stage renal disease. When diabetic patients present with massive proteinuria, physicians often assume DKD as the underlying cause. However, some patients decline renal biopsy, increasing the risk of undiagnosed coexisting glomerular diseases. Clinical data indicated that diabetic patients had higher age, blood glucose levels, and HbA1C levels compared to other groups. Nevertheless, these parameters alone were insufficient to confirm the pathology of DKD or distinguish it from other glomerular diseases. Similarly, although proteinuria levels were elevated in other glomerular diseases, no data confirmed their specificity to any particular disease[26–28]. Previous studies have explored the discordance between pathological findings and clinical markers, including proteinuria levels[29].

Previous studies have also suggested that, despite the pathophysiological differences between DKD and primary glomerular disease—primarily resulting from an immunologic process—both conditions can lead to podocytopathy and subsequent proteinuria. One study reported that the severity of proteinuria in primary glomerular disease ranged from 1.5 to 10 g per day, making it indistinguishable from proteinuria caused by DKD without a renal biopsy[29]. Despite attempts to use alternative diagnostic methods, such as detecting rs1888747 polymorphisms in the FRMD3 gene, these approaches have demonstrated limitations in reliably distinguishing DKD from other glomerular diseases[5]. Additionally, urinary podocin[30] has been found to be more effective in predicting disease severity rather than differentiating DKD from primary glomerular diseases.

This study aimed to differentiate DKD from other primary glomerular diseases using VOC testing, a method previously employed in diagnosing various cancers[16,31]. The approach was based on the hypothesis that diseases with distinct pathophysiology would generate unique VOC patterns when interacting with different metal oxides. A protocol adapted from previous research by the same group was utilized, focusing on variables that could be readily implemented in future practical applications. These included the minimum electrical resistance and the time required for electrical resistance to decrease from the initial point to the minimum during VOC exposure to electrical heat.

Furthermore, this study highlights a paradigm shift in VOC research, demonstrating that VOC-based diagnostics are not solely limited to oncological applications but may extend to metabolic and renal disorders. The findings reinforce the hypothesis that VOCs possess disease-specific signatures, supporting their potential to serve as diagnostic biomarkers across various medical conditions.

Literature has identified several targeted VOCs associated with diabetes-related diseases, including 2-aminothiazoline-4-carboxylic acid, N-acetyl-S-(2-carboxyethyl)-L-cysteine, N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine, N-acetyl-S-(3-hydroxypropyl)-L-cysteine, N-acetyl-S-(2-hydroxypropyl)-L-cysteine, phenyl glyoxylic acid, and N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine[15]. These findings align with the results of this study, which demonstrated significant differences in semiconductors S2 and S3 (out of five semiconductors) due to their specificity in detecting VOCs such as hydrogen, ethanol, hydrogen sulfide, and ammonia. The VOCs associated with diabetes-related diseases shared structural characteristics, including ethanoyl (acetyl), hydrogen, and aromatic amines, which were detectable by these semiconductors.

The ability to differentiate DKD from other primary glomerular diseases using semiconductors did not rely on the specificity of any single substance. Instead, it stemmed from the cumulative VOCs of each disease and their properties in electron capture and release within the chamber during changes in heat energy. The results for hydrogen and ethanol VOCs demonstrated that patients with other causes of NS exhibited higher electrical resistance changes compared to normal individuals but lower than those observed in diabetic patients. Diabetic patients showed a distinct increase in electrical resistance, and when DKD-induced proteinuria was present, VOCs caused a significant decrease in electrical resistance with heat energy release. However, throughout the experiment, diabetic patients consistently exhibited higher electrical resistance than normal individuals.

This study proposed a novel approach for distinguishing DKD from other primary glomerular diseases by assessing the overall pathophysiology rather than relying on a single biomarker, thereby collectively measuring the VOC properties. Diagnostic specificity was further evaluated using ROC curves, which demonstrated that VOCs detected by semiconductor S2, particularly hydrogen and ethanol, effectively differentiated disease stages (DM vs. DM with DKD) and distinguished DM with DKD from other NS. However, the AUC in Fig. 1B, which declined when assessing the differentiation between DM with and without DKD, indicated reduced diagnostic performance, despite remaining statistically significant. This finding underscored that DM served as the underlying pathology, leading to shared VOC characteristics between the two groups, as both originated from the same primary disease. This was the key point intended to be conveyed. Additionally, the study excluded diabetic patients with concurrent NS, emphasizing the need for future research to compare histological changes. This exclusion raised concerns that VOCs associated with NS could potentially interfere with those linked to diabetes, thereby reducing the accuracy of the ROC analysis.

This study had two main limitations. First, as an observational cohort, it did not include renal biopsy for DM patients with proteinuria less than 1 gram/day. Although patients with albuminuria < 30 mg/day were carefully selected as the DM without DKD group for clarity, the possibility remained that some patients with undiagnosed DKD were inadvertently included.

Second, in the group with other causes of nephrotic syndrome, no patients had concurrent diabetes, raising concerns about real-world scenarios where primary NS might coexist with DM. Such coexistence could alter VOC patterns and potentially reduce the accuracy of ROC analysis.

The cohort size was determined based on the exploratory nature of the study, focusing on a specific patient population to ensure clarity in group classification. Furthermore, as a proof-of-concept study, this research did not investigate the specific biological mechanisms linking VOCs to disease pathophysiology, highlighting the need for future studies to enhance understanding. These limitations should be addressed in future research to improve both scientific validity and clinical applicability.

Conclusion

Diagnosing VOCs in diabetic patients, with or without proteinuria from DKD, using semiconductors targeting hydrogen and ethanol successfully distinguished these patients from normal individuals and those with other NS. This study served as a proof of concept, demonstrating the feasibility of extending VOC analysis beyond cancer diagnostics to identify and differentiate metabolic diseases. The results further suggest that VOC biosensors hold promise as a versatile diagnostic tool applicable to a wide range of medical conditions, beyond their initial oncological applications. These findings introduced a novel framework for disease diagnosis by leveraging overall VOC patterns and biomarker properties, aligning with approaches previously explored in various cancers.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

Chatchai Kreepala developed the theory, performed the computations and worked out almost all of the technical details. Vichayut Suthat Na Ayutaya performed the experiments. Rungrueang Phatthanakun verified the analytical methods and supervised the findings of this work. Chatchai Kreepala, Kittipong Thabsuwan, and Jirayupa Paewponsong wrote the draft of manuscript. All authors discussed the results and contributed to the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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