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COMPARING THE ROLE OF URINARY HEME OXYGENASE-1 AND URINARY ALBUMIN AS BIOMARKERS FOR DIABETIC NEPHROPATHY

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ABSTRACT

Background: Diabetic nephropathy (DN) may cause end-stage renal disease. Prompt detection and management are crucial for slowing the progression of DN and improving patient outcomes. Traditional diagnostic methods, such as assessing albuminuria and serum creatinine levels, frequently fail to detect early kidney damage, as structural changes in the kidneys can occur before any rise in albumin excretion. Urinary heme oxygenase-1 (UHO-1), a cytoprotective enzyme induced by oxidative stress, has emerged as a potential early biomarker for DN. **Aim**: This study aims to investigate whether urinary UHO-1 levels can serve as an earlier biomarker of DN and compare the results with those of the conventional markers of DN. **Material and methods:** A cross-sectional study was conducted on 85 participants with type 2 diabetes (45 diabetics and 40 with DN) at the Diabetes Center in Ajdabiya, Libya, from May to August 2024. Clinical assessments and laboratory tests, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), serum creatinine, blood urea, urine albumin, albumin-creatinine ratio (ACR), and urinary HO-1 (UHO-1), were performed. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS). **Results:** UHO-1 levels were not significantly ($p = 0.547$) different in DN patients compared to diabetics. In contrast, common markers such as ACR and microalbuminuria showed significant increases in DN patients ($p < 0.001$). When DN patients were further classified according to ACR, urinary HO-1 showed a mild but non-significant ($p > 0.05$) increase across these subgroups. Whereas the classical markers of DN (microalbuminuria, ACR, GFR, urinary creatinine, and albumin) were significantly different in DN groups compared to normal albuminuric subjects. However, UHO-1 levels in the macroalbuminuric group showed a strong negative correlation ($r = -0.994$) with HbA1c, moderate correlation ($r = -0.566$) with blood urea, and weak correlation with other renal function markers (serum creatinine and urinary albumin). group, indicating that UHO1 levels is inversely related to glycemic control and the degree of renal damage. Conversely, the gold biomarkers (microalbuminuria and ACR) were significantly changed among DN patients with different stages. **Conclusion:** Urinary HO-1 may be regarded as a non-inferior, compared to the other classical biomarker of diabetic nephropathy.

KEYWORDS: Chronic kidney disease, diabetic nephropathy, heme oxygenase-1, microalbuminuria, macroalbuminuria.

INTRODUCTION

Diabetes is a serious health issue affecting people globally. The incidence of diabetes has grown considerably across the world, with recent estimates indicating that over 537 million adults are affected (Asamoah et al., 2019).

Diabetic nephropathy (DN) is a long-term condition that progresses over several years. It is commonly marked by a slow rise in urinary albumin excretion, elevated blood pressure, an increased risk of cardiovascular disease, and a declining glomerular filtration rate (GFR), ultimately resulting in renal failure that is treated by dialysis or renal replacement therapy [Tang et al., 2016].

Oxidative stress is one of the key factors contributing to the pathophysiology of diabetic nephropathy. Oxidative stress, caused by an imbalance between oxidants and antioxidants, is a significant factor in DN's development and is often a result of hyperglycemia. Elevated reactive oxygen species (ROS) resulting from hyperglycemia contribute to increased pro-inflammatory proteins, leading to inflammation (Bahia et al., 2006).

HO is a key enzyme in heme catabolism, catalyzing the conversion of heme into biliverdin, carbon monoxide (CO), and iron ($Fe2^{+}$). There are 3 subtypes of HO; the first is an inducible "HO-1", whose concentration increases in response to O_2 stress, the second is a constitutive "HO-2", and the third one is HO-3, whose role is not fully understood, and was first found (Grunenwald et al., 2021). However, they serve as scavengers of free radicals and effective fighters against inflammation, thus combating the damage resulting from oxidative stress. (Nath 2006).

HO has anti-inflammatory, antioxidant, anti-apoptotic, and immunomodulatory properties that support cell homeostasis. Normally, its expression is low in healthy tissues but increases with ischemia, hypoxia, injury, toxins, and other harmful stimuli. This change helps convert an oxidative environment into an antioxidant one, aiding tissue repair. It is believed to be essential in preventing acute kidney injury and slowing the progression of chronic renal disease (M. Nath & Agarwal, 2020).

Researchers found that levels of HO-1 in serum and urine rise as renal damage worsens, and the severity of renal damage is reduced in situations accompanied by a lack of HO-1. Zager and Johnson, 2010.

In 2023, Zhai and Wu, elegantly reviewed the expression of HO-1 in many renal cells. The expression of HO-1 in the kidneys varies significantly. The epithelium tissue lining of the kidney tubule exhibits the highest capacity to produce the HO-1 during acute kidney injury (AKI). In cases of DN, HO levels in podocytes show a notable increase, whereas their expression in glomeruli is comparatively lower. Nevertheless, the reason behind this differential expression remains unclear, and it is suggested to be connected to the specific location of renal damage. Furthermore, the stimulation of HO-1 synthesis through its substrates and various pharmaceutical agents may offer therapeutic benefits. Numerous medications have demonstrated positive outcomes in enhancing HO-1 expression and fostering kidney conservation in presymptomatic studies. Substances like heme and its derivatives, metal inducers, pharmaceutical agents, natural stimulators, and byproducts of the HO enzyme are crucial, as prompt intervention can slow the decline in kidney function and reduce negative outcomes.

Microalbuminuria is recognized as the earliest indicator of the onset of DN. Nevertheless, a significant percentage (about 62%) of individuals with type 2 diabetes may experience kidney damage in advance of the appearance of albumin in urine, which is the main tool for earlier detection of DN (Tang et al. 2016). Additionally, there are numerous confounding factors related to albuminuria, including physical activity, urinary tract infections, acute illnesses, and heart failure. Moreover, it has been observed that nondiabetic individuals can also excrete albumin in their urine, demonstrating the lack of specificity of albuminuria for accurately predicting diabetic kidney disease. Rigalleau et al., 2007. UHO-1 is present in urine before the onset of microalbuminuria. Therefore, we aim to investigate the hypothesis that urinary heme oxygenase 1 serves as a more sensitive marker than albumin for the early detection of DN.

MATERIALS AND METHODS

Patients and Participants: 85 type 2 diabetic patients, divided into 2 groups; 45 had diabetes without DN, and 40 had diabetes with DN, were enrolled in this cross-sectional study. All patients were recruited at the Diabetes Treatment Center in Ajdabiya city, located in the Eastern part of Libya. Between May 2024 and August 2024. All participants gave informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Benghazi Medical Hospital in Benghazi.

Exclusion criteria: Diabetic subjects were not included in this research if they suffered from ketonemia or episodes of hypoglycemia in the last 90 days before the beginning of the study. Patients are also excluded if they have urinary system disorders (blood in urine, urinary tract infection, cancer, glomerulus inflammation, renal stones, or urinary system conditions other than DN), disease of the other body systems or any other hormonal disorder. The study also excluded subjects suffering from cardiac or circulatory disorders, and patients who take drugs for hypertension, or antihyperlipidemic therapy in the last 6 months prior the start of the study.

Blood tests: Blood samples were taken from all patients, and some necessary tests were performed, which were fasting blood sugar tests (FBG) by the Celltac Alpha device, glycosylated hemoglobin tests (HbA1c) by the Epithod 616, and kidney function tests (Blood Urea, Serum Creatinine) using the Cobas Integra 400 device.

Urine tests: the samples were collected from all subjects in the early morning. Then, some tests were performed, such as albumin, creatinine, and albumin/creatinine ratio (ACR mg/g), by the Afinion 2 Analyzer. And for microalbuminuria, the estimated glomerular filtration rate (eGFR) is estimated from the modification of diet in renal disease (MDRD) formula (Mendivil et al. 2023) as follows.

eGFR (ml/min/1.73 m2) = 186 × [serum creatinine (mg/dl)]-1.154×[age]-0.203× [0.742 if female] × [1.21 if male]

The rest of the sample was then prepared for freezing. The urine specimens were poured into aseptic disposable tubes and instantly separated using a centrifuge for five minutes at refrigerator temperature. The upper sediment was discarded and stored at -20°C for future urinary emeoxygenase-1 (uHO-1) ELISA testing (Human ELISA slide size HO1: ELISA kit 96T, AFG Bioscience).

Statistical analysis: All data obtained from the study were analyzed and presented using the Statistical Package for Social Sciences (SPSS) version 26, developed by IBM. Numeric variables were presented as mean and standard deviation (SD), whereas categorical variables were expressed as numbers and percentages. The mean values of continuous variables were compared in both groups using the Non-parametric Statistical test (Mann-Whitney U) to assess whether the means of test parameters were statistically different between the study groups. The ANOVA test is used to calculate the significance among the 3 subgroups of the study. The parametric test, specifically the t-test, was used to analyze the results of the UHO1 test in both groups. We have also used Pearson's correlation coefficient to test the correlation between the continuous variables.

RESULTS

Distribution of the study population

85 type 2 diabetic patients (aged 40 to 80 years), divided into two groups: 45 had diabetes without DN, and 40 had only diabetes. as Table 1describes the gender and age of both groups.

Table 1: shows the gender, numbers, and percentages of diabetics in the current study.

Gender	Study Groups		Total
	Diabetics	Diabetes with DN	
Male	20 (23.5%)	18 (21.2%)	38 (44.7%)
Female	25 (29.4%)	22 (25.9%)	47 (55.3%)
Total	45 (52.9%)	40 (47.1%)	85 (100%)

Type 2 diabetes patients were further divided into three subgroups according to the ACR value, as shown in Table 2.

Table 2: shows the distribution of the study population according to the ACR values.

ACR Levels	Study Groups	
	Diabetics	Diabetics with DN
Normoalbuminuric group ACR < 30 mg/g (Without DN)	45 (100)	(25%)10
Microalbuminuric group ACR ≤ 300 mg/g (early DN)	0 (0%)	27 (67.5%)
Macroalbuminuric group ACR ≥ 300 mg/g (Obvious DN)	0 (0%)	(7.5%)3
Total	(100%)45	(100%)40

Comparing the study parameters in the diabetic group and the DN group

The mean level of HbA1c of DN groups was significantly elevated compared with that of the diabetic group (Table 3), whereas FBG levels showed no significant variation between the study groups.

On the other hand, the mean level of UHO1 was slightly but not significantly increased in the DN group compared with that of the diabetic group. In the meantime, classical biomarkers of DN; The values of microalbuminuria, ACR, and urinary albumin of diabetic nephropathy patients showed a highly significant elevation compared with those of the diabetic group (Table 3).

In a similar vein to that of UHO1, blood urea, serum creatinine, urinary creatinine and eGFR values of the DN group were non-significantly changed compared to those of the diabetic group as clear the following table.

Table 3: Comparison of laboratory findings in the diabetic group versus DN group.

Test	Diabetic Group (Mean ± SD)	DN Group (Mean ± SD)	t-value	Z-value	p-value
FBS	172 ± 62.85	205.3 ± 82.74	-	-1.760	0.078
HbA1C	7.2 ± 1.69	7.81 ± 1.54	-	-2.079	0.038
Blood Urea	19.3 ± 7.31	22.20 ± 10.27	-	-1.404	0.160

Serum Creatinine	0.64 ± 0.20	0.75 ± 0.499	-	-.831	0.406
Urinary albumin	10.63 ± 6.94	104.1 ± 76.57	-	-7.692	0.000
Microalbuminuria.	11.36 ± 5.82	261.2 ± 440.9	-	-7.661	0.000
Urinary Creatinine	134.8 ± 68.2	136.0 ± 84.71	-	-.431	0.666
ACR	9.32 ± 5.38	115.7 ± 130.89	-	-7.603	0.000
eGFR	105.7 ± 12.6	98.22 ± 21.077	-	-1.851	0.064
UHO1	19.45 ± 1.72	19.68 ± 1.71	-.0605	-	0.547

(**Note**: all of the study parameters were analyzed using the Mann-Whitney test; except the UHO1 test was analyzed by the t-student test. The red colour means significant differences).

The comparison of the mean value of the eGFR of the diabetic group was non-significantly decreased when compared to that of the DN group as shown in table. 3.

Correlation between the study parameters

By examining the correlation analysis between the studied biochemical parameters, several positive and negative relationships were observed.

The UHO1 levels showed low inverse (negative) relationships with all the studied parameters. On the other hand, Blood urea exhibited a strong positive correlation with serum creatinine (r = 0.795), indicating a direct physiological relationship between them, while it showed moderate positive correlations with urinary albumin and weak correlations with ACR, microalbuminuria, and HO-1. Similarly, serum creatinine also demonstrated moderate positive correlations with urinary albumin and weak correlations with ACR and microalbuminuria (Table 4).

Furthermore, Table 4 shows that urinary albumin has a moderate to strong positive correlation with the classical markers of DN: ACR and microalbuminuria, indicating that these renal biomarkers increase together as nephropathy progresses. A weak positive correlation was also found between ACR and microalbuminuria.

Table 4: The relation between the study tests represented by the correlation coefficient.

Control Variables		FBG	HbA1c	B.urea	S.Creat.	U.Creat.	U.Alb.	ACR	Microalb.	HO1	GFR
FBG	Correlation	1.000	.655	.109	-.023	.038	-.001	.020	.041	-.128	.058
	Significance (2-tailed)	.	.000	.340	.840	.741	.990	.862	.721	.260	.615
	df	0	77	77	77	77	77	77	77	77	77
HbA1c	Correlation	.655	1.000	.133	-.045	-.039	.093	.024	.100	-.038	.060
	Significance (2-tailed)	.000	.	.242	.691	.734	.413	.831	.381	.737	.601
	df	77	0	77	77	77	77	77	77	77	77
B. Urea	Correlation	.109	.133	1.000	.795	-.126	.361	.267	.189	.015	-.733
	Significance (2-tailed)	.340	.242	.	.000	.270	.001	.017	.095	.896	.000
	df	77	77	0	77	77	77	77	77	77	77
S. Creat.	Correlation	-.023	-.045	.795	1.000	-.130	.398	.296	.215	-.032	-.874
	Significance (2-tailed)	.840	.691	.000	.	.255	.000	.008	.057	.779	.000
	df	77	77	77	0	77	77	77	77	77	77
U. Creat.	Correlation	.038	-.039	-.126	-.130	1.000	-.052	-.403	.010	-.098	.202
	Significance (2-tailed)	.741	.734	.270	.255	.	.650	.000	.929	.392	.074
	df	77	77	77	77	0	77	77	77	77	77
U. Alb.	Correlation	-.001	.093	.361	.398	-.052	1.000	.608	.759	-.018	-.382
	Significance (2-tailed)	.990	.413	.001	.000	.650	.	.000	.000	.876	.001
	df	77	77	77	77	77	0	77	77	77	77
ACR	Correlation	.020	.024	.267	.296	-.403	.608	1.000	.286	-.038	-.276
	Significance (2-tailed)	.862	.831	.017	.008	.000	.000	.	.010	.741	.014
	df	77	77	77	77	77	77	0	77	77	77
Microalb	Correlation	.041	.100	.189	.215	.010	.759	.286	1.000	-.064	-.281
	Significance (2-tailed)	.721	.381	.095	.057	.929	.000	.010	.	.574	.012
	df	77	77	77	77	77	77	77	0	77	77

	HO1	Correlation	-.128	-.038	.015	-.032	-.098	-.018	-.038	-.064	1.000	-.030
		Significance (2-tailed)	.260	.737	.896	.779	.392	.876	.741	.574	.	.795
		df	77	77	77	77	77	77	77	77	0	77
	GFR	Correlation	.058	.060	-.733	-.874	.202	-.382	-.276	-.281	-.030	1.000
		Significance (2-tailed)	.615	.601	.000	.000	.074	.001	.014	.012	.795	.
		df	77	77	77	77	77	77	77	77	77	0

Note: FBG: Fasting Blood Glucose, HbA1c: Glycosylated hemoglobin, B. Urea: Blood Urea, S. Creat.: Serum Creatinine, U. Creat.: Urinary Creatinine, U. Alb.: Urinary Albumin, ACR: Albumin-Creatinine ratio, Microalb: Microalbuminuria, HO1: Urinary Heme Oxygenase 1, GFR: Glomerular Filtration Rate.

Results of the study subgrouping

Type 2 diabetes patients were further divided into 3 groups according to the ACR values, as shown in Table 2: Normoalbuminuric, Microalbuminuric, and Macroalbuminuric groups.

Significant differences (Table 5) were observed in Urinary Creatinine, Urinary albumin, and GFR observed in the comparison of the macroalbuminuric with the microalbuminuric and the normoalbuminuric groups. On the other hand, no significant difference in the levels observed in the comparison of UHO1, FBG, HbA1c, blood urea, and serum creatinine.

Table 5: Comparing study parameters among the 3 subgroups of the study.

Test	**Subgroup 1** (Mean ± SD)	**Subgroup 2** (Mean ± SD)	**Subgroup 3** (Mean ± SD)	**F-value**	**P-value**
FBS	184 ± 75 mg%	196 ± 72 mg%	192 ± 104 mg%	0.263	0.791
HbA1C	7.3 ± 1.7 %	7.7 ± 1.5 %	7.9 ± 2.2 %	0.571	0.567
Blood Urea	19.0 ± 7 mg%	23 ± 11 mg%	23.7 ± 12 mg%	1.831	0.167
Serum Creatinine	0.6 ± 0.2 mg%	0.8 ± 0.6 mg%	0.9 ± 0.5 mg%	2.637	0.078
Urinary albumin	17 ± 17 mg	119 ± 79 mg	165 ± 68 mg	49.798	0.000
Microalbuminuria.	21 ± 31 mg	333 ± 519 mg	376 ± 382 mg	11.057	0.000
Urine Creatinine	148 ± 79 mg%	120 ± 62 mg%	35 ± 14 mg%	4.306	0.017
eGFR	106±12ml/min	96 ± 21 ml/min	89 ± 38 ml/min	3.997	0.022
UHO1	19 ± 1.7 ng/ml	19 ± 1.7 ng/ml	19 ± 1.9 ng/ml	0.001	0.999

Note: Subgroup 1: Normoalbuminuric, Subgroup 2: Microalbuminuric, and Subgroup 3: Macroalbuminuric.

Correlation of UHO1 and the study parameters in the 3 subgroups of the study

There were Strong negative correlations (Pearson's Correlation) shown only in the subgroup 3 (macroalbuminuric) between UHO1 and FBS (r = -0.837), and also with HbA1c (r = –0.994). Moreover, a moderate negative correlation between UHO1 and b. urea (r = –0.566). A weak negative correlation was observed between UHO1 and s. creatinine and urinary albumin, but these findings cannot be generalized due to the small number of subjects in subgroup 3, as is clear in Table 6.

Table 6: Correlation of UHO1 and other parameters in the subgroups of the study.

Subgroups (based on ACR)		**FBS**	**HbA1C**	**B. Urea**	**S. Creat**	**U. Creat**	**U. alb**	**Micralb.**	**GFR**
Subgroup 1 HO1	P. Correlation	-0.101	0.035	0.034	0.058	-0.18	0.002	0-.124	-0.040
	Sig. (2-tailed)	0.467	0.802	0.808	0.675	0.189	0.986	0.368	0.773
	N	54	54	54	54	55	55	55	54
Subgroup 2 HO1	P. Correlation	-0.172	-0.067	0.077	-.072	0.014	0.091	-0.054	0-.102
	Sig. (2-tailed)	0.390	0.740	0.703	.723	0.944	0.653	0.805	0.613
	N	27	27	27	27	27	27	23	27
Subgroup 3 HO1	P. Correlation	-0.837	-0.994	-0.566	-0.247	0.197	-0.28	-0.021	0.140
	Sig. (2-tailed)	0.368	0.068	0.617	0.841	0.874	0.821	.0987	0.910
	N	3	3	3	3	3	3	3	3

Note: FBG: Fasting Blood Glucose, HbA1c: Glycosylated hemoglobin, B. Urea: Blood Urea, S. Creat.: Serum Creatinine, U. Creat.: Urinary Creatinine, U. Alb.: Urinary Albumin, ACR: Albumin-Creatinine ratio, Microalb: Microalbuminuria, HO1: Urinary Heme Oxygenase 1, GFR: Glomerular Filtration Rate.

DISCUSSION

In the current study, the mean value of UHO1 (19.7 ± 1.7 ng/dl) in the diabetic nephropathy patients was non-significantly (p= 0.547) different from the mean level of UHO1 (19.4 ± 1.72 ng/dl) of the diabetic group without nephropathy. Our finding contradicts a recent study by Aboeleil et al. (2023), who investigated 40 normoalbuminuric patients (ACR less than 30 mg/g), 40 microalbuminuric patients (ACR 30–300 mg/g), and 20 healthy control subjects. Aboeleil et al. found a significant elevation (P < 0.001) in UHO-1 levels (5.02) compared with normoalbuminuric patients (3.01) and controls (0.3). The latter evidence demonstrated that the elevation in the concentrations of UHO-1 in the microalbuminuric subjects preceded the presence of albuminuria. The author also concluded that the UHO1/creatinine ratio could be used to confirm the diagnosis of diabetic nephropathy. On the same line with Li et al. 2017 demonstrated that elevated levels of UHO-1 were observed in type 2 diabetics before the development of marked albuminuria, and are linked to renal impairment. The author also suggested the use of UHO-1 for early detection of renal damage in diabetes.

Our research also disagreed with the findings of Abdelhameed and Saied in 2019, in which the UHO-1 levels in the microalbuminuric group showed a highly significant increase ($P < 0.000$) compared with the UHO-1 levels in the normoalbuminuric group and control group.

In the meantime, the present study found a highly significant increase in urinary albumin levels (104 ± 76 mg/l) in the DN group compared with urinary albumin (10.6 ± 6.9 mg/l) in the group including diabetics without DN. Similar findings are observed in the other renal outcome parameters, including microalbuminuria and ACR. Moreover, urinary creatinine was significantly ($p<0.017$) increased in the macroalbuminuric group compared with the microalbuminuric and normoalbuminuric groups. These findings confirmed the role of microalbuminuria and ACR as the gold biomarkers of DN. Furthermore, our study has suggested a possible role of urinary albumin and creatinine as additional markers for DN. These results are consistent with the work of Karimi and colleagues (2024), who suggested that a combination of both serum and urinary biomarkers could improve diagnostic precision and allow earlier treatment for DN.

On the other hand, urinary HO-1 in the macroalbuminuric subgroup was negatively correlated (r = –0.994) with HbA1c, indicating that higher glycemic burden was associated with lower urinary HO-1 levels. Our findings suggested that oxidative stress in nephropathic diabetics overcomes the antioxidant role exerted by UHO1. On the contrary, Aboeleil et al. 2023 showed no significant correlation between UHO-1 and HbA1c. However, in a recent study, da Fonseca et al. 2021 demonstrated a role of HO-1 in situations of hyperglycemia.

Similarly, the current study showed a moderate negative correlation ($r = –0.566$) between HO-1 and blood urea. These inverse relationships suggest that as diabetic nephropathy progresses to the advanced macroalbuminuric stage, the expression of HO-1 decreases, possibly reflecting reduced antioxidant role or cytoprotective response in the face of increased oxidative stress that accompanies severe renal damage.

Although no statistically significant difference in UHO-1 levels was observed between the diabetic and nephropathy groups using an independent t-test, a moderately significant negative correlation between UHO-1 and blood urea in the macroalbuminuric subgroup, as well as a weak negative correlation with urinary albumin and serum creatinine, was seen in Table 6. Furthermore, a strong significant association between UHO1 levels with HbA1c levels (r = -0.999) and also with FBG (r = - 0.564), but these findings cannot be generalized due to the very low number of subjects in the macroalbuminuric subgroup. These inverse relationships suggest that while HO-1 may not differ markedly between study groups and subgroups, it is closely related to the degree of renal damage and glycemic control.

While the literature presents conflicting findings regarding HO-1 as a diagnostic biomarker, some studies have suggested a protective role against renal oxidative injury. Guijarro-Munoz et al. 2014 demonstrated that UHO-1 levels were negatively associated with eGFR and albuminuria; these findings were consistent with our work, in which we found no negative correlation between the levels of UHO-1 and eGFR. Notably, Jiang et al. 2010 reported an inverse association between HO-1 and HbA1c, suggesting that poor glycemic control may suppress HO-1 expression and exacerbate kidney stress. These findings, also in agreement with our own, conclude that the levels of UHO-1 may reflect the burden of oxidative stress and early metabolic imbalance, rather than serving as a consistent biomarker for early detection of renal damage and DN.

The strong negative correlation between urinary HO-1 and HbA1c, as well as the moderate negative correlation with blood urea observed in the macroalbuminuric subgroup, indicates that HO-1 expression may decline with worsening metabolic control and renal impairment. This inverse relationship is suggested to reflect a poor antioxidant defense system in advanced nephropathy, where persistent oxidative stress leads to cellular damage resulting from the unsuitable compensatory capacity of HO-1.

Study limitations

The study failed to find a clear difference in HO-1 levels in the different stages of diabetic kidney disease; this might be due to the small sample size of the study

population. The reason behind these limitations is the limitations of the laboratory and research resources. In addition, the results of the present work cannot be generalized due to the lack of standardization of the test method for testing UHO-1.

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