# Role of kidney injury molecule 1 and nephrin as biomarkers for diagnosis of nephropathy in type 2 diabetes mellitus

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**Background**
Diabetic nephropathy (DN) is a significant complication of diabetes caused by alterations within the structure and function of the kidneys. This increases the need for novel biomarkers that might predict nephropathy. Kidney injury molecule 1 (KIM-1) is a type 1 membrane protein present on the apical membrane of proximal tubules. It has a possible role in predicting long-term renal outcome. Thus, it serves as a selected and sensitive biomarker for proximal tubule damage. Nephrin is a transmembrane protein in the structure of the slit diaphragm. The study aimed to assess the levels of urinary KIM-1 and nephrin to detect early changes of renal functions in patients with type 2 diabetes mellitus (T2DM) and to assist in the prevention.

**Patients and methods**
This is a prospective study comprising 60 patients with T2DM. Patients were divided into three groups by their urinary albumin/creatinine ratio. Peripheral hemogram, liver and renal functions, lipogram, glycosylated hemoglobin (HbA1C), urine albumin/creatinine ratio, urinary KIM-1, and nephrin were done. Patients with type 1 DM, fever, infection, gestational diabetes, as well as evidence of systemic disease were excluded. Moreover, 28 volunteers were included.

**Results**
In this study, urinary nephrin and KIM-1 were significantly higher in those with macroalbuminuria, microalbuminuria, and those with normoalbuminuria compared with the control group. Both nephrin and KIM-1 had a significant positive correlation with creatinine in patients with macroalbuminuria and patients with microalbuminuria. Multivariate logistic regression analysis showed that the odds ratio for the presence of DN in the highest KIM-1 was 3.01 (95% confidence interval = 2.11–5.60; $P < 0.001$), nephrin was 2.9 (95% confidence interval = 1.10–4.65; $P < 0.001$), and HbA1C was 2.23 (95% confidence interval = 1.94–4.11; $P < 0.001$). By using receiver operating characteristic, it was noticed that the level of nephrin with cutoff value of more than 10 μg/ml was able to detect the diagnosis and prognosis of DN in our patients with sensitivity of 95%, specificity of 94%, and positive predictive value of 98.2%.

**Conclusion**
Urinary KIM-1 and nephrin levels appear to increase in kidney injury secondary to DN in the early period regardless of albuminuria, as urinary KIM-1 and nephrin were increased, even though there was normal urinary albumin excretion in the normoalbuminuric group. The study revealed that KIM-1, nephrin, and HBA1C were independent predictors of DN.

**Keywords:**
Diabetic nephropathy, glycosylated hemoglobin, kidney injury molecule 1 and nephrin, type 2 diabetes mellitus

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## Introduction

Diabetic nephropathy (DN) is a condition that develops over several years and is characterized by a gradual increase in urinary albumin secretion. DN is among the severe complications that occur in diabetic patients and results in an elevated hazard of death owing to all causes, cardiac disease, and development of renal damage [1]. There is considerable evidence that early management could prevent the progress of the disorder [2]. Moreover, data indicate that most patients with macroalbuminuria can revert to normoalbuminuria, and the idea of nonalbuminuric DN is recognized, reflecting that diabetic patients can present with a decrease within glomerular filtration rate (GFR) without advancing from normoalbuminuria to macroalbuminuria [3]. Biomarkers provide an active and dominant approach to know the range of a disease from the earliest manifestations to the terminal stage [4]. Microalbuminuria is known to be the first marker of DN; still, a huge fraction of kidney damage occurs during a nonalbuminuric state or before the start of microalbuminuria [3].

Kidney injury molecule 1 (KIM-1) is a type 1 membrane protein present on the apical membrane proximal tubules [5]. It has a potential role in predicting long-term renal outcome [6]. Damage of the slit diaphragm results in nephrinuria. Nephrinuria was stated in some normoalbuminuric diabetic patients [7].

Nephrin is a renal glomerular filtration barrier protein that is an important constituent of the slit diaphragm, which constitutes the size-selective filter of the kidney [8]. Presence of nephrin in urine is connected to podocyte damage, representing a biomarker of early glomerular damage [9]. Dysregulation of nephrin in podocytes in DN may result in nephrinuria in normoalbuminuric patients, before microalbuminuria [10].

## Patients and methods

The current study enrolled 60 patients with type 2 diabetes mellitus (T2DM) who were subdivided into three groups according to the urinary albumin/creatinine ratio. Group 1 included 20 patients with T2DM with macroalbuminuria, group 2 included 25 patients with T2DM with microalbuminuria, and group 3 included 15 patients with T2DM with normoalbuminuria. In addition, 28 apparently healthy volunteers were included. The patients were selected from the outpatient clinic of Internal Medicine Department of Assiut University Hospital in the period from January 2019 to June 2019.

Sample collection, storage, and handling:

(1) Random blood sample: 8 ml of venous blood was collected under complete aseptic conditions and divided into the following:
   (a) Four milliliters of venous blood was collected into two EDTA containing tubes: one for measuring glycosylated hemoglobin and another for complete blood count.
   (b) Four milliliters was collected into a plain tube without anticoagulant.
      (i) Blood was allowed to clot for 10–20 min at room temperature and then centrifuged at the speed of 2000–3000 rpm for 20 min.
      (ii) The collected serum was inspected to ensure that it is clear and shows no hemolysis for measuring blood glucose, kidney functions, liver functions, and lipid profile.
(2) Morning urinary samples were collected: one part for urinary albumin-creatinine ratio and the other part for measuring both urinary nephrin and KIM-1. Urinary KIM-1 was measured by sandwich ELISA technique catalog No: 10180 Sino Geneclon Biotech (Hangzhou, China). Urinary nephrin was measured by sandwich ELISA technique catalog No. 10513, Sino Geneclon Biotech.

### Statistical analysis

Data were analyzed via SPSS (Statistical Package for the Social Sciences, version 20; IBM, Armonk, New York, USA). Continuous data were expressed in the form of mean ± SD, whereas nominal data were expressed in the form of frequency (percentage). $\chi^2$ test was used to compare the nominal data of different groups, whereas Student *t* test was used to compare the means of two different groups and analysis of variance test for more than two groups. Pearson correlation was used to assess the correlation coefficient of different variables with nephrin and KIM-1. Multivariate regression analysis was used to detect different predictors for early renal impairment in patients with DM. Receiver operating characteristic curve was used to determine the performance of KIM-1 and nephrin in early prediction of nephropathy in patients with T2DM. Level of confidence was kept at 95%, and *P* value was significant if less than 0.05.

### Ethical consideration

Formal consent was obtained from patients and controls. The study was accepted by the Ethical Committee of Faculty of Medicine, Assiut University.

## Results

The practical part of the current study was performed at the Clinical Pathology Department of Assiut University Hospitals. It was performed between January 2019 and June 2019. The study enrolled 60 patients with T2DM who were subdivided into three groups according to the urinary albumin/creatinine ratio: patients with macroalbuminuria (20 patients) (33.3%), patients with microalbuminuria (25 patients) (41.7%) and patients with normoalbuminuria (15 patients) (25%). The study included 28 healthy subjects as control. The aim of the study was to assess the role of KIM-1 and nephrin as biomarkers for early diagnosis of nephropathy in T2DM (Tables 1 and 2).

Fig. 1 shows that KIM-1 had an insignificant correlation with all other parameters in different groups with the exception of a significant positive correlation with creatinine in patients with macroalbuminuria ($r = 0.42$; $P = 0.01$).

In Figs. 2 and 3, it was noticed that nephrin had an insignificant correlation with all other parameters in

**Table 1 Baseline data of studied groups**

| | Macroalbuminuria (*n*=20) | Microalbuminuria (*n*=25) | Normoalbuminuria (*n*=15) | Control group (*n*=28) | *P*1 | *P*2 | *P*3 | *P*4 | *P*5 | *P*6 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 54.35±7.54 | 52.8±7.93 | 54.67±9.88 | 50.11±9.90 | 0.57 | 0.91 | 0.28 | 0.53 | 0.6 | 0.27 |
| Sex [*n* (%)] | | | | | 0.8 | 0.89 | _ | 0.35 | 0.8 | 0.89 |
| Male | 10 (50) | 17 (68) | 8 (53.3) | 14 (50) | | | | | | |
| Female | 10 (50) | 8 (32) | 7 (46.7) | 14 (50) | | | | | | |
| Hemoglobin (g/dl) | 9.74±0.70 | 10.28±1.64 | 11.07±1.59 | 12.93±1.18 | 0.18 | 0.01 | 0.01 | 0.07 | 0.01 | 0.01 |
| Leukocytes ($10^3$/ml) | 9.07±2.70 | 8.45±2.42 | 7.34±1.34 | 8.38±3.02 | 0.34 | 0.06 | 0.36 | 0.19 | 0.91 | 0.13 |
| Platelets ($10^3$/ml) | 267.95±89.44 | 297.32±97 | 258.40±95.55 | 310.64±99.10 | 0.31 | 0.77 | 0.13 | 0.22 | 0.62 | 0.09 |
| LDL (mg/dl) | 94.10±20.72 | 89.16±38.16 | 72.53±30.14 | 88.11±38.46 | 0.62 | 0.06 | 0.54 | 0.13 | 0.91 | 0.15 |
| HDL (mg/dl) | 50.75±13.81 | 42.56±12.74 | 39.86±11.75 | 43.81±12.21 | 0.13 | 0.14 | 0.06 | 0.51 | 0.72 | 0.33 |
| TG (mg/dl) | 102.55±37.57 | 120.88±75.46 | 115.60±60.56 | 119.60±41.93 | 0.27 | 0.49 | 0.30 | 0.77 | 0.93 | 0.82 |
| Cholesterol (mg/dl) | 167.70±22.76 | 155.24±49.46 | 144.06±40.03 | 149.25±49.86 | 0.34 | 0.11 | 0.15 | 0.43 | 0.61 | 0.71 |
| RBS (mmol/l) | 15.13±3.39 | 14.68±2.32 | 13.17±3.69 | 5.85±0.96 | 0.56 | 0.03 | 0.01 | 0.07 | 0.01 | 0.01 |
| HbA1C (%) | 7.95±1.92 | 7.74±1.67 | 7.49±2.26 | 4.88±0.76 | 0.67 | 0.41 | 0.01 | 0.63 | 0.01 | 0.01 |

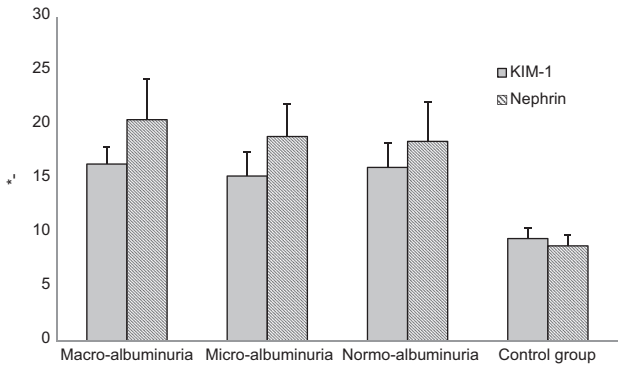
Data were expressed as *r* (strength of correlation) and *P* (significance of correlation). *P* value was significant if less than 0.05. Cr, creatinine; HbA1C, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RBS, random blood sugar; TG, triglyceride.

**Table 2 The mean urinary levels of kidney injury molecule 1 and nephrin in studied patients with type 2 diabetes mellitus**

| Parameters | Macroalbuminuria (*n*=20) | Microalbuminuria (*n*=25) | Normoalbuminuria (*n*=15) | Control group (*n*=28) | *P*1 | *P*2 | *P*3 | *P*4 | *P*5 | *P*6 |
|---|---|---|---|---|---|---|---|---|---|---|
| Urinary KIM-1 (ng/ml) | 16.39±1.62 | 15.28±2.24 | 16.08±2.27 | 9.64±1.57 | 0.57 | 0.63 | 0.01 | 0.21 | 0.01 | 0.01 |
| Urinary nephrin (µg/l) | 20.50±3.78 | 18.94±2.99 | 18.50±3.61 | 8.81±1.03 | 0.07 | 0.64 | 0.01 | 0.64 | 0.01 | 0.01 |
| Urinary KIM-1/creatinine | 0.03±0.01 | 0.02±0.011 | 0.02±0.01 | 0.003±0.001 | 0.46 | 0.41 | 0.01 | 0.51 | 0.01 | 0.01 |
| Urinary nephrin/creatinine | 0.03±0.01 | 0.02±0.021 | 0.02±0.01 | 0.002±0.001 | 0.38 | 0.12 | 0.01 | 0.11 | 0.01 | 0.01 |

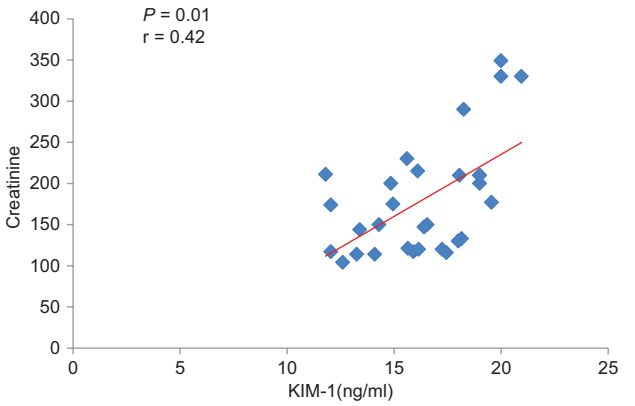
KIM-1, kidney injury molecule 1. *P*1 comparison between macroalbuminuria and microalbuminuria. *P*2 comparison between macroalbuminuria and normoalbuminuria. *P*3 comparison between macroalbuminuria and control group. *P*4 comparison between microalbuminuria and normoalbuminuria. *P*5 comparison between microalbuminuria and control group. *P*6 comparison between normoalbuminuria and control group.

**Figure 1**




The mean urinary KIM-1 and nephrin levels in studied subjects. KIM-1, kidney injury molecule 1.

**Figure 2**



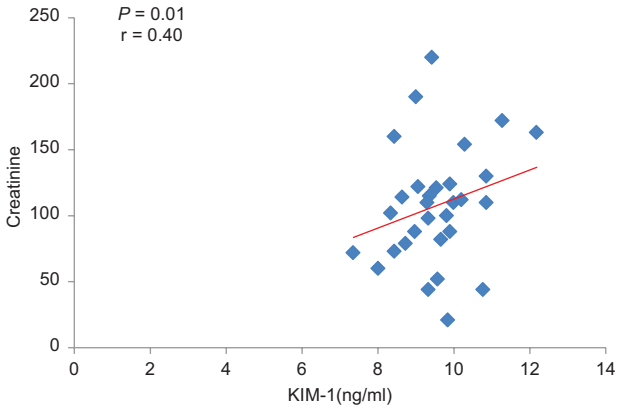

Correlation between urinary KIM-1 and creatinine in the macroalbuminuria group. KIM-1, kidney injury molecule 1.

different groups with the exception of a significant positive correlation with creatinine in patients with macroalbuminuria ($r$ = 0.41; $P$ = 0.01) and those with microalbuminuria ($r$ = 0.35; $P$ = 0.03) (Fig. 4 and Tables 3 and 4).

It was noticed that nephrin had an insignificant correlation with all other parameters in different groups, with the exception of a significant positive correlation with creatinine in the macroalbuminuria group ($r$ = 0.41; $P$ = 0.01) and those with microalbuminuria ($r$ = 0.35; $P$ = 0.03) (Fig. 5).

(1) Patient groups were subdivided based on eGFR into the following: stage I with eGFR more than 90 ml/min [6/60 (10%)], stage II with eGFR ranging from 60 to 89 ml/min [36/60 (60%)], and stage III ranging from 30 to 59 ml/min [18/60 (30%)], as shown in Table 5. Patients with stage III had significantly lower hemoglobin compared with stage I (10.48 ± 1.5 vs. 11.41 ± 1.81 g/dl; $P$ < 0.001) and stage II (9.55 ± 0.67 vs. 11.41 ± 1.81 g/dl; $P$ = 0.02).
(2) Moreover, patients with stage III had significantly higher urea and creatinine compared with stage

**Figure 3**




Correlation between urinary KIM-1 and creatinine in the microalbuminuria group. KIM-1, kidney injury molecule 1.

**Table 3 Accuracy of kidney injury molecule 1 and nephrin in prediction of diabetic nephropathy in studied patients**

| ROC | KIM-1 (%) | Nephrin (%) |
|---|---|---|
| Sensitivity | 43.3 | 95 |
| Specificity | 100 | 94 |
| Positive predictive value | 100 | 98.2 |
| Negative predictive value | 43 | 87.1 |
| Accuracy | 61 | 94 |
| Cutoff point | >12 | >10 |
| Area under curve | 0.78 | 0.83 |
| *P* | <0.001 | <0.001 |

KIM-1, kidney injury molecule 1; ROC, receiver operating characteristic. *P* value was significant if less than 0.05.

**Table 4 Predictors of early renal impairment in patients with diabetes**

| Predictors | Univariate OR (95% CI) | *P* | Multivariate OR (95% CI) | *P* |
|---|---|---|---|---|
| Age | 2.34 (1.19-4.50) | <0.001 | – | – |
| HbA1C | 3.45 (2.10-3.99 | <0.001 | 2.23 (1.94-4.11) | <0.001 |
| KIM-1 | 4.50 (3.33-6.70) | <0.001 | 3.01 (2.11-5.60) | <0.001 |
| Nephrin | 2.11 (1.20-4.50) | <0.001 | 2.90 (1.10-4.65) | <0.001 |

95% CI, 95% confidence interval; HbA1C, glycosylated hemoglobin; KIM-1, kidney injury molecule 1; OR, odds ratio. *P* value was significant if less than 0.05.

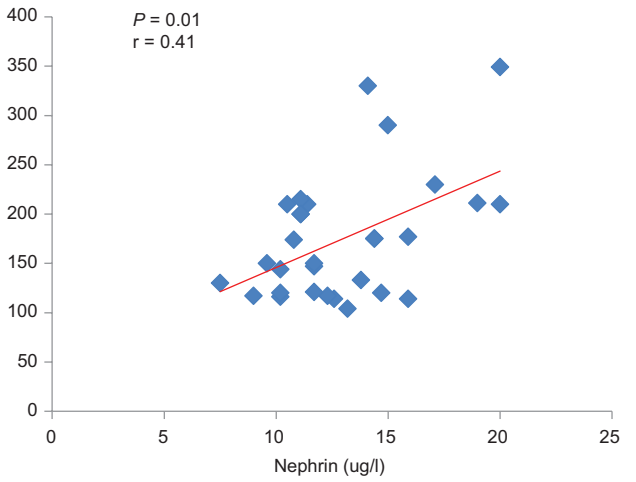
I (11.82 ± 6.62 vs. 7.21 ± 2.16 mmol/l; $P < 0.001$) and stage II (11.82 ± 6.62 vs. 9.65 ± 5.84 mmol/l; $P < 0.001$), respectively.

(3) Creatinine clearance was significantly higher in patients with stage I compared with stage III (86.48 ± 15.45 vs. 71.34 ± 11.37 ml/min, respectively; $P = 0.04$).

(4) Patients with stage III had a significantly higher albumin-creatinine ratio compared with stage I(332.67±90.82vs.93.30±34.56mg/g,respectively; $P < 0.001$) and stage II (332.67 ± 90.82 vs. 134.34 ± 25.89 mg/g, respectively; $P = 0.02$).

(5) The different stages of chronic kidney diseases (CKD) had insignificant differences regarding urinary KIM-1 and nephrin ($P > 0.05$).

**Figure 4**




Correlation between mean levels of urinary nephrin and creatinine in the macroalbuminuria group.

## Discussion

DN is a common consequence of diabetes [11] and is defined as an increase in the urinary albumin excretion rate and impaired renal function [12]. The event of DN arises over a period of 10–20 years, ranging from microalbuminuria and advancing to end-stage renal impairment [13]. Microalbuminuria is an indicator of DN and a predictor of its development. However, data suggest the inability of microalbuminuria changes to predict nephropathy advancement [14].

The research community is concentrating on various approaches to improve the sensitivity of biomarkers to expect patients who will develop DN or are in danger of developing renal impairment [15].

This study enrolled 60 patients with T2DM divided into three groups based on the urinary albumin/creatinine ratio: patients with macroalbuminuria (20 patients) (22.8%), microalbuminuria (25 patients) (28.4%), and normoalbuminuria (15 patients) (17%). In addition, 28 apparently healthy subjects were enrolled as control (31.8%).

The study showed that the hemoglobin level was significantly higher in patients with normoalbuminuria compared with those with macroalbuminuria, probably as the level of albuminuria is increased, the prevalence of anemia also increased. Our results are consistent with Priya [16], who showed a higher prevalence of anemia in diabetic population and that the prevalence of nephropathy was higher in diabetic patients with low hemoglobin level.

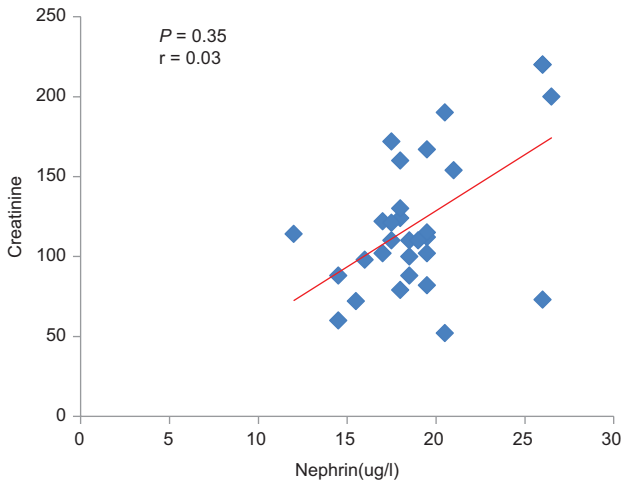
Chronic diseases, such as DM, are related to mild-to-moderate anemia called anemia of

**Table 5 Data of studied patients based on stages of chronic kidney disease**

| | Stage I (*n*=6) | Stage II (*n*=36) | Stage III (*n*=18) | *P*1 | *P*2 | *P*3 |
|---|---|---|---|---|---|---|
| Age (years) | 51±7.45 | 53.77±8.77 | 54.72±7.53 | 0.45 | 0.34 | 0.69 |
| Sex [*n* (%)] | | | | 0.92 | 0.94 | 0.93 |
| Male | 3 (50) | 15 (41.7) | 8 (44.4) | | | |
| Female | 3 (50) | 21 (58.3) | 10 (55.6) | | | |
| Hemoglobin (g/dl) | 11.41±1.81 | 10.48±1.53 | 9.55±0.67 | 0.12 | <0.001 | 0.002 |
| RBS (mmol/l) | 13.53±2.48 | 14.08±3.22 | 15.50±2.97 | 0.68 | 0.18 | 0.12 |
| HbA1C (%) | 7.57±2.50 | 7.81±1.75 | 7.68±2.04 | 0.76 | 0.89 | 0.80 |
| Creatinine (µmol/l) | 76.33±19.02 | 82.52±13.58 | 94.94±11.97 | 0.31 | <0.001 | <0.001 |
| Urea (mmol/l) | 7.21±2.16 | 9.65±5.84 | 11.82±6.62 | 0.35 | <0.001 | <0.001 |
| CrCL (ml/min) | 86.48±15.45 | 77.78±18.63 | 71.34±11.37 | 0.23 | 0.04 | 0.18 |
| eGFR (ml/min/1.73 $m^2$) | 99.66±11.39 | 69.63±6.33 | 57.33±4.70 | <0.001 | <0.001 | <0.001 |
| Alb/creatinine (mg/g) | 93.30±34.56 | 134.34±25.89 | 332.67±90.82 | 0.41 | <0.001 | <0.001 |
| KIM-1 | 15.97±2.96 | 17.80±2.19 | 19.98±1.64 | 0.85 | 0.95 | 0.85 |
| Nephrin | 18.83 ± 5.87 | 18.98 ± 2.98 | 21.85 ± 3.55 | 0.58 | 0.96 | 0.96 |

Alb/Cr, albumin/creatinine ratio; Cr, creatinine; CrCL, creatinine clearance; eGFR, estimated glomerular filtration rate; HbA1C, glycosylated hemoglobin; KIM-1, kidney injury molecule 1; RBS, random blood sugar. *P*1 comparison between stage I and stage II. *P*2 comparison between stage I and stage III. *P*3 comparison between stage II and stage III.

**Figure 5**




Correlation between mean levels of urinary nephrin and creatinine in the microalbuminuria group.

inflammation, which is in consistent with a study by Khandare *et al.* [17], who found that the normal group had a significantly higher hemoglobin level than diabetic groups.

Patients with macroalbuminuria had significantly higher random blood sugar in comparison with those with normoalbuminuria, microalbuminuria, and control group, indicating that poor glycemic control contributes to progression of albuminuria. Similarly, another study found that random blood glucose and glycosylated hemoglobin levels were significantly higher in diabetic patients with normoalbuminuria and microalbuminuria compared with the control group [18].

In this study, the control group had significantly lower urinary nephrin in comparison with those with macroalbuminuria, those with microalbuminuria, and those with normoalbuminuria. Nephrin had a significant positive correlation with creatinine in patients with macroalbuminuria and those with microalbuminuria. This is similar to a study by Jim *et al.* [19], who found that all patients with macroalbuminuria and microalbuminuria had elevated nephrin.

In a study done on patients with T2DM, nephrinuria was related to decreased levels of eGFR, even in normoalbuminuric patients, therefore signifying that nephrinuria could be associated with the progression of renal impairment in the stage of normoalbuminuria. Our results are consistent with the fact that patients with T2DM and normoalbuminuria are considered as a group of patients at a lower risk of developing CKD [20].

In early type 2 diabetes, decreased levels of nephrin can be detected despite other currently used markers like albumin are not detected. Large amounts of nephrin and albumin are excreted in urine along with elevated levels of urea and creatinine in blood [21].

In this study, there was a significant increase in urine KIM-1 in patients with microalbuminuria and macroalbuminuria than patients with normoalbuminuria and control. Moreover, KIM-1 had a significant positive correlation with creatinine in patients with macroalbuminuria and patients with microalbuminuria in this study. Our result was similar to the studies of Garg *et al.* [22] and Petrica *et al.* [7], who found that urine KIM-1 levels were increased in patients with microalbuminuria and macroalbuminuria than those with normoalbuminuria. The elevation of KIM-1 in normoalbuminuric, microalbuminuric, and macroalbuminuric groups than the control group is a result of damage of proximal tubules with excretion

of KIM-1 in urine during tubular injury, making it detectable in the urine of diabetics.

A notable finding is that in studies of human biopsies with tubular necrosis or atrophic damage, KIM-1 is not produced. The authors presumably conclude that KIM-1 may be a worthy marker of active tubular damage and not tubular scarring [23].

In this study, urinary KIM-1/creatinine was significantly lower in the control group compared with those with macroalbuminuria, microalbuminuria, and normoalbuminuria.

In consistent with this study, a cross-sectional study to investigate the prevalence of tubular damage in the early stage (<5 years) of type 2 diabetes showed that levels of urinary KIM-1 were significantly elevated in the diabetic group compared with the control group [24].

In this study, urinary KIM-1 and levels were increased throughout the stages of CKD without a significant difference between them. We believe that the cause of these findings is the development of tubular atrophy and fibrosis, which limited the marked expression of nephrin and KIM-1 throughout the advancement of the CKD stages. This is in agreement with a study by Tekce *et al*. [25].

Nephrin with a cutoff value of more than 10 μg/ml had a total expected probability of 94% in patients with DN. Our results are similar to a study by Kostovska *et al*. [26], showing that nephrin has a total expected probability of 96% in subjects with DN. This suggests high discriminatory power among healthy subjects and patients with DN; similarly, this result means high sensitivity and specificity of nephrin as a urinary biomarker in the early detection of DN.

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Nil.

**Conflicts of interest**
None declared.

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