Al-Azhar International Medical Journal

Volume 5 | Issue 7 Article 9

7-31-2024
Section: Internal Medicine

Urinary Cadherin E a Marker for Early Detection of Diabetic Nephropathy

Fawzy Hamed Hassan Saafan
Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt

Mohamed Ahmed El Sayed Ahmed
Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt

Ahmed Ali Ali Assem
Clinical Pathology, Faculty of Medicine for boys, Al-Azhar University, Cairo, Egypt

Mahmoud Adel Mohamed Ahmed Ghoneim
Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt,
mimoadel242@gmail.com

How to Cite This Article

Saafan, Fawzy Hamed Hassan; Ahmed, Mohamed Ahmed El Sayed; Assem, Ahmed Ali Ali; and Ghoneim, Mahmoud Adel Mohamed Ahmed (2024) "Urinary Cadherin E a Marker for Early Detection of Diabetic Nephropathy," *Al-Azhar International Medical Journal*: Vol. 5: Iss. 7, Article 9.
DOI: https://doi.org/10.58675/2682-339X.2528

ORIGINAL ARTICLE

Urinary Cadherin E a Marker for Early Detection of Diabetic Nephropathy

Fawzy H. H. Saafan [a], Mohamed A. E. Ahmed [a], Ahmed A. A. Assem [b], Mahmoud A. M. A. Ghoneim [a,*]

[a] Department of Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt
[b] Department of Clinical Pathology, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt

Abstract

Background: ***Diabetic nephropathy (DN) is a prevalent consequence of diabetes as well as the primary factor contributing to the development of chronic kidney disease. It is a degenerative condition characterized by the gradual deterioration of kidney function over time, ultimately resulting in end-stage renal disease (ESRD).***

Aim and objectives: ***To examine the function of Urine E cadherin as an indicator for the early identification of diabetic nephropathy.***

Patients and methods: ***This prospective trial was done on 100 persons admitted to the Nephrology outpatients for diabetic nephropathy at Damanhur Teaching Hospital. Based on the Diabetic nephropathy (DN) stage, the patients were separated into four groups: Stage 1 DN group included 24 cases, the Stage 2 DN group included 27 cases, the Stage 3 DN group included 25 cases, and Stage 4 DN group included 24 cases.***

Results: ***There was statistically significant variance amongst the participated groups Regarding Urinary sE-cadherin/Cr (mg/l), glycated hemoglobin & serum creatinine ($p<0.05$) while there was no statistically significant change amongst the studied groups as regard duration also type of diabetes, diastolic blood pressure (DBP), systolic blood pressure (SBP), heart rate, temperature, fasting glucose, respiratory rate, urine albumin-to-creatinine ratio (UACR), albuminuria, glycated hemoglobin, glomerular filtration rate (GFR) blood urea nitrogen (BUN), & urine creatinine ($p>0.05$).***

Conclusion: ***The present investigation establishes E-cadherin as a newfound indicator for diabetic nephropathy, which might be employed to ascertain the progression of DN. Consequently, evaluating the levels of E-cadherin in diabetic individuals could potentially have a significant effect on decelerating or possibly preventing end-stage renal disease.***

Keywords: Urinary cadherin E; Diabetic nephropathy; ESRD

1. Introduction

Diabetic nephropathy (DN) is a prevalent consequence of diabetes as well as the primary contributor to the development of chronic kidney disease. According to estimates from 2015, the global prevalence of diabetes mellitus exceeded 415 million individuals. By the year 2040, the total number of individuals will rise to 642 million, with 90 percent of them being who have type 2 diabetes.[1]

Diabetic nephropathy is defined by persistent elevation of albumin levels in urine as well as being linked to a significant risk of cardiovascular morbidity and mortality. DN is a degenerative condition characterized by the gradual deterioration of kidney function, ultimately resulting in end-stage renal disease. Although there have been improvements in the management and treatment of diabetes as well as its complications, diabetic nephropathy remains a prominent cause of end-stage renal disease globally. This emphasizes the necessity for more efficient diagnostic, prognostic, and therapeutic approaches.[2]

The Cadherins, a group of transmembrane proteins, primarily contribute to cell adhesion and signaling. They function in a calcium-dependent manner. Research has demonstrated that Cadherins are vital in increasing renal epithelial polarization. This is achieved through their interaction with neighboring cells via the extracellular domains of Cadherins, which share the same molecular structure.[3,4]

Accepted 21 July 2024.
Available online 31 July 2024

* Corresponding author at: Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt.
E-mail address: mimoadel242@gmail.com (M. A. M. A. Ghoneim).

https://doi.org/10.58675/2682-339X.2528

Of the fifty cadherins, the most well-studied ones are type one, specifically E-cadherin. The adherent junction of epithelial cells is home to the transmembrane protein E-cadherin.[5] E-cadherin is highly prevalent in the distal tubules. At the same time, it is either absent or present in deficient levels in the proximal tubules. The involvement of E-cadherin in the assessment regulation of kidney transplantation is vital. During the initial phases of EMT, there is a downregulation of E-cadherin expression, leading to the dissociation of cells within the epithelial layer.[6]

This trial aimed to examine the function of Urine E cadherin as an indicator of timely diagnosis of diabetic nephropathy.

2. Patients and methods

This prospective research was performed on 100 cases admitted to the Nephrology outpatients for diabetic nephropathy at Damanhur Teaching Hospital. Based on the Diabetic nephropathy (DN) stage, the patients were divided into 4 groups: Stage 1 DN group included 24 cases, Stage 2 DN group included 27 cases, Stage 3 DN group included 25 cases, and Stage 4 DN group included 24 cases.

Ethical approval: Both the study protocol and the written informed consent were approved by the Local Ethics Committee.

Inclusion criteria: Patients with type 1 and 2 periods of DM for more than ten years and a normal albumin-creatinine ratio.

Exclusion criteria: Individuals with the following conditions: diabetic kidney disease, heart failure, present or recent infection, chronic liver disease, allergy condition, acute or chronic inflammatory illness, cancer, autoimmune disease, and end-stage renal disease.

Sample Size: This trial base on a study done by Koziolek et al.,1 The sample size was calculated using Epi Info STATCALC, taking into account the following assumptions: A confidence level of 95% is used, with a power of 80 percent for both sides. The estimated odds ratio has an error margin of 5%, resulting in a value of 1.115. The ultimate maximum sample size extracted from the Epi-Info output was 93. Therefore, the sample size was augmented to 100 individuals to account for potential drop-out instances throughout the follow-up period.

Methods:

All patients underwent:

Complete history taking, general examination, laboratory investigations, and urine analysis for cadherin E.

Sample Collection and Handling

Midstream urine was collected in 15 mL tubes and centrifuged at 1000× g for 10 min at 4 °C to remove cell debris and casts. The supernatant was aliquoted into 2 mL aliquots and used immediately or stored at –80 °C until use. From each collected urine sample, we used 2 mL to measure routine laboratory parameters and we measure urine cadherin E in urine.

HbA1C % assay was done using Crystal Chem's hemoglobin A1C kit.

Principle of the assay: The Hemoglobin A1c kit utilized an enzymatic assay method, where lysed whole blood samples underwent thorough protease digestion. This mechanism liberated amino acids, including glycated valines, from the beta chains of hemoglobin. Glycated valines after that act as substrates for the particular fructose valine oxidase (FVO) enzyme. The FVO enzyme preferentially hydrolyzes N-terminal valines and generates hydrogen peroxide as a byproduct. The measurement used a horseradish peroxidase (POD) catalyzed reaction and an appropriate chromagen. No additional measurement of total Hemoglobin (Hb) was required in this enzymatic HbA1c assay.

Assay procedures: As required, we introduced 112 µL of Reagent CC1a and 48 µL of Reagent CC1b into every microplate well. We thoroughly mixed the contents by repeatedly pipetting. For each well, we introduced 25 µL of lysate from either a sample, calibrator, or control and thoroughly mixed the contents through repeated pipetting. We placed the microplate in an incubator set at a temperature of 37ºC and allowed it to reach thermal equilibrium for 5 minutes. We quantified absorbance by employing a plate reader to measure A700 values. We introduced 70 µL of Reagent CC2 and thoroughly mixed it by pipetting repeatedly. We quantified the rise in absorbance at 37ºC for three minutes using a plate reader (measuring A700 values).

The sandwich-ELISA technique assessed E-cadherin utilizing the human E-Cadherin ELISA Kit (Elabscience, China).

Test Principle: The micro ELISA plate included in this kit was pre-coated with a Human E-Cad-specific antibody. The micro ELISA plate wells were supplemented with standards or samples and mixed with the corresponding antibody. Subsequently, a detection antibody that is biotinylated and specific to Human E-Cad and Avidin-Horseradish Peroxidase (HRP) conjugate was sequentially introduced into each well of the microplate and incubated. The components were liberated and carried away by water. The substrate solution was introduced into every well. Wells that exhibited a blue color were exclusively those that contained biotinylated detection antibody, Human E-cadherin, Avidin-HRP conjugate. The enzyme-substrate reaction was halted by introducing a stop solution, resulting in a blue coloration. The OD was quantified using spectrophotometry at a wavelength of 450 nm ± 2 nm. The optical density

value directly correlates with the concentration of Human E-Cadherin (E-Cad). The optical density of the samples was contrasted with the standard curve to quantify the concentration of Human E-Cadherin in the samples.[7]

Assay procedures: We introduced 100µL of either a standard or a sample into the wells and subjected them to incubation for 90 minutes at a temperature of 37°C. The liquid was removed, then 100µL of Biotinylated Detection Ab working solution was promptly inserted into each well. The samples were incubated at 37°C for 60 minutes. We performed three aspirations and washed on the plate. We introduced 100 microliters of HRP conjugate working solution and allowed it to incubate for 30 minutes at 37 degrees Celsius. We performed aspiration and washing of the plate five times. We introduced 90 microliters of Substrate Reagent as well as incubated it for 15 minutes at a temperature of 37 degrees Celsius. We included 50 microliters of Stop Solution. We promptly measured the plate's absorbance at a wavelength of 450nm.

3. Results

Table 1 exhibited that there was statistically no significant amongst studied groups in relation to gender, age & MBI (p>0.05).

Table 1. Distribution of demographic data amongst both groups.

		STAGE 1 DN N =24	STAGE 2 DN N =27	STAGE 3 DN N = 25	STAGE 1 DN N =24	P VALUE
AGE MEAN±SD		55.6±7.5	57.7±6.59	57.28±7.09	56.8±8.13	0.76
SEX	Male	12 (50%)	13 (48.1%)	13 (52%)	13 (54.2%)	0.9
	female	12 (50%)	14 (51.9%)	12 (84%)	11 (45.8%)	
BMI MEAN±SD		30.06±5.1	30.6±4.5	32.4±7.6	30.5±5.2	0.49

P value <0.05 statistically significant

Table 2 displayed that Statistical analysis revealed no significant distinction across the groups with respect to duration & type of diabetes (p>0.05).

Table 2. Distribution of medical history among the examined groups.

		STAGE 1 OF DN NO (24)	STAGE 2 OF DN NO (27)	STAGE 3 OF DN NO (25)	STAGE 4 OF DN NO (24)	P VALUE
DURATION OF DIABETES MEAN ± SD		24.41±10.16	29.33±7.49	27.6±8.43	29.04±8.76	0.1850
TYPE OF DIABETES	Type 1	8(33.3%)	9(33.3%)	9(36%)	7(29.2%)	0.9666
	Type 2	16(66.6%)	18(66.6%)	16(64%)	17(70.8%)	

Table 3 indicated that No statistically significant variation was seen amongst the groups that were tested when it came to diastolic blood pressure, systolic blood pressure, heart rate, temperature & respiratory rate. (p>0.05)

Table 3. Distribution of vital signs among studied groups.

	STAGE 1 DN N =24	STAGE 2 DN N =27	STAGE 3 DN N = 25	STAGE 1 DN N =24	P VALUE
SBP (MMHG)	134.6± 5.9	134.3± 3.3	134.7± 6.4	136.6± 6.07	0.45
DBP (MMHG)	91.85 ±13.2	90.6 ±11.6	96.4± 7.4	88.8 ±12.7	0.11
TEMPERATURE	37.4± 0.67	37.2 ±0.44	37.2± 0.4	37.3± 0.5	0.46
HEART RATE(BEAT/MIN)	93.5 ±22.7	97.2± 22.4	99.7± 19.1	98.04 ±19.7	0.76
RESPIRATORY RATE(BREATH/MIN)	18.45± 4.6	20.5 ±4.57	19.84 ±5.01	19.04± 4.95	0.44

Table 4 revealed that Between the groups that were examined, no statistically significant disparity was identified in terms of fasting glucose, glycated hemoglobin, urine albumin-to-creatinine ratio , albuminuria, blood urea nitrogen , glomerular filtration rate & urine creatinine (p>0.05), while there was statistically variance among the studied groups as regard glycated hemoglobin and serum creatinine (p<0.05).

Table 4. Distribution of Clinical data between the studied groups.

	STAGE 1 OF DN NO (24)	STAGE 2 OF DN NO (27)	STAGE 3 OF DN NO (25)	STAGE 4 OF DN NO (24)	P VALUE
FASTING GLUCOSE(MMOL/L)	9.5± 0.5	9.51± 0.63	9.6± 0.7	9.62± 0.5	0.43
GLYCATED HEMOGLOBIN (%) MEAN ± SD	8.255±1.12	7.85±1.12	7.48±1.13	8.32±1.06	0.0319
UACR(MG/G) MEAN ± SD	22.16±1.80	22.06±1.40	21.65±1.77	21.50±1.37	0.4075
ALBUMINURIA(MG/L) MEAN ± SD	349.56±322.22	285.84±174.40	353.85±194.37	454.37±331.36	0.1581
SERUM CREATININE (µMOL/L) MEAN ± SD	94.60±20.91	88.44±6.77	89.97±7.06	100.33±20.22	0.0291
BUN (MMOL/L) MEAN ± SD	7.29 ±0.58	7.51±0.72	7.33±0.54	7.25±0.60	0.4437

EGFR ML/MIN MEAN ± SD	60.66±10.26	58.75±2.89	58.75±3.01	57.48±10.03	0.5153
URINE CREATININE (MG/L) MEAN ± SD	9.76±0.89	10.27±1.43	9.61±1.19	9.55±0.80	0.0889

Table 5 showed that Statistical analysis revealed no discernible variation across the groups under consideration with respect to pallor, cyanosis, jaundice & lymph node enlargement (p>0.05).

Table 5. Distribution of signs & symptoms amongst the studied groups.

	STAGE 1 OF DN NO (24)	STAGE 2 OF DN NO (27)	STAGE 3 OF DN NO (25)	STAGE 4 OF DN NO (24)	P VALUE
PALLOR					
YES	21(87.5%)	24(88.88%)	23(92%)	21(87.5%)	0.9523
NO	3(12.5%)	3(11.12%)	2(8%)	3(12.5%)	
CYANOSIS					
YES	21(87.5%)	24(88.88%)	23(92%)	21(87.5%)	0.9523
NO	3(12.5%)	3(11.12%)	2(8%)	3(12.5%)	
JAUNDICE					
YES	21(87.5%)	24(88.88%)	23(92%)	21(87.5%)	0.9523
NO	3(12.5%)	3(11.12%)	2(8%)	3(12.5%)	
LYMPH NODE ENLARGEMENT					
YES	18(75%)	22(81.48%)	22(88%)	20(83.33%)	0.6961
NO	6(25%)	5(18.52%)	3(12%)	4(16.66%)	

Table 6 displayed that there was statistically significant alteration amongst the examined groups regarding Urinary sE-cadherin/Cr (mg/l) (p<0.05).

Table 6. Distribution of Urinary sE-cadherin/Cr (mg/l) between the studied groups.

	STAGE 1 OF DN NO (24)	STAGE 2 OF DN NO (27)	STAGE 3 OF DN NO (25)	STAGE 4 OF DN NO (24)	P VALUE
URINARY SE-CADHERIN/CR (MG/L) MEAN ± SD	874.67±116.79	798.86±108.17	832.90±129.49	907.57±120.07	0.0091

4. Discussion

Regarding demographic data, the current trial initiated no significant connotation among DN stages with age, sex, or BMI (p>0.05).

In concordance with the current study, Mohamed et al.[8] showed no significant alteration among the normoalbuminuric DM, micro-albuminuric DM, macro-albuminuria DM, control groups regarding age and sex.

However, Salman et al.,[9] revealed that type II diabetic patients with macro-albuminuria were significantly older than those with microalbuminuria and no albuminuria. However, there was no significant variance as regards sex and BMI. The contrast with the current study regarding age may be related to sample size differences.

The present trial results demonstrated no statistical association between DN stage and DM duration or type (p>0.05).

In concordance with the current study, Badawy et al.[10] reported no significant relationship between the duration of diabetes and the stages of diabetic nephropathy.

In disagreement with the present research, El-Dawla et al.,[7] revealed that there was a significant relation between DN stage and DM duration.

Our results showed no statistically variance among studied groups regarding SBP, DBP, temperature, heart rate, and respiratory rate. (p>0.05)

This comes in agreement with El-Dawla et al.,[7] who initiated no significant alterations in systolic or diastolic blood pressure among diabetic groups. Similar results were reported by Soliman et al.,[11].

In contrast, Thiet al.[12] showed that patients with macroalbuminuria have significantly higher SBP DBP compared to those with microalbuminuria, normoalbuminuria, and control groups.

Regarding fasting glucose, glycated hemoglobin, UACR, albuminuria, BUN, GFR, and urine creatinine, our results indicated no statistical distinction among the groups. However, regarding glycated hemoglobin and serum creatinine, there was a statistically significant variance (p<0.05).

In line with our study, Mohamed et al.[8] presented a highly significant alteration in HbA1C amongst the normoalbuminuric DM, microalbuminuria DM, macro-albuminuria DM, and control groups.

Also, Abdelwahid et al.,[13] showed that glycosylated hemoglobin was significantly higher in diabetic patients with microalbuminuria (9.3 ± 2.2) and macro-albuminuria (10.5 ± 2.3) than in those with an average Alb/Cr ratio (8.3 ± 1.9%).

Regarding the distribution of signs and symptoms amongst the studied groups, the current study revealed that there was non-statistically significant variance between the studied groups according to pallor, cyanosis, jaundice, and lymph node enlargement.

To the best of our knowledge, no studies in the literature have assessed the differences in signs and symptoms between the diabetic groups of different DN stages.

The above results highlighted the high need for biomarkers to detect DN and differentiate different stages of DN. Urinary sE-cadherin was suggested to be a potential marker for early detection of diabetic nephropathy.

In the current study, there was significant change amongst the studied groups according to Urinary sE-cadherin/Cr (mg/l), supposing the potential utility of Urinary sE-cadherin/Cr as an early marker for diabetic nephropathy progression.

Consistent with the findings of this research, Koziolek et al.,[1] proved that E-cadherin in urine may be used to identify diabetic kidney damage early on in a long-term study. Not only did they observe that E-cadherin levels are higher in nephropathy individuals, but they could also distinguish amongst the various phases of DN. They went so far as to say that the increase in urine E-cadherin levels was noticed 20 ± 12.5 months prior to the start of microalbuminuria.

Consistent with the present evaluation, Aly et al.,[14] discovered that microalbuminuria was associated with an elevated urinary sE.cadherin/cr level, as opposed to the control and normoalbuminuria groups. The researchers concluded that urinary sE.cadherin levels could serve as a useful indicator for detecting microalbuminuria as well as renal impairment in those with type 2 diabetes who otherwise have normoalbuminuria.

Limitations: The current study was limited by being a single-center study, having a small sample size, and having a relatively short follow-up period.

4. Conclusion

Based on the latest research, E-cadherin, a new marker for diabetic nephropathy, can be used to identify the stage of the condition. These results demonstrate that epithelial-mesenchymal transition plays a role in DN development and progression. Thus, measuring E-cadherin levels in diabetes patients may be useful in reducing the rate of end-stage renal disease or maybe even preventing it altogether.

Future comparison research should use bigger samples and longer follow-up periods to validate our findings and uncover risk factors for end-stage renal disease.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

Funding

No Funds : Yes

Conflicts of interest

There are no conflicts of interest.

References

1. Koziolek M, Mueller GA, Dihazi GH, et al. Urine E-cadherin: A Marker for Early Detection of Kidney Injury in Diabetic Patients. J Clin Med. 2020;9(3):639.
2. Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. Am J Kidney Dis. 2014;63(2 Suppl 2):S39-S62.
3. van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci. 2008;65(23):3756-3788.
4. Aberle H, Schwartz H, Kemler R. Cadherin-catenin complex: protein interactions and their implications for cadherin function. J Cell Biochem. 1996;61(4):514-523.
5. Brüser L, Bogdan S. Adherens Junctions on the Move-Membrane Trafficking of E-Cadherin. Cold Spring Harb Perspect Biol. 2017;9(3):a029140.
6. Lenferink, A. E. Epithelial-to-Mesenchymal transition (EMT): the good, the bad, and the ugly. In Cancer Systems Biology .CRC Press..2010; pp. 233-260.
7. El-Dawla NMQ, Sallam AM, El-Hefnawy MH, El-Mesallamy HO. E-cadherin and periostin in early detection and progression of diabetic nephropathy: epithelial-to-mesenchymal transition. Clin Exp Nephrol. 2019;23(8):1050-1057.
8. Mohamed EA, Morsy AA, Mohamed HT, Alrahim A, Mohammed N. Predictive Roles of Urinary Liver Type-Fatty Acid-Binding Protein and N-Acetyl-β-D-Glucosaminidase for Progression of Diabetic Nephropathy in Type 2 Diabetic Patients. The Egyptian Journal of Hospital Medicine. 2020 Jan 1;78(1):9-20.
9. Salman AA, Salman MA, Said M, et al. Albuminuria as a predictor of mortality in type II diabetic patients after living-donor liver transplantation. Ann Med. 2022;54(1):2598-2605.
10. Badawy Othman AI, Elalem A, Mohamed S, Elsherbini D, Riad NA. Association Between Diabetic Nephropathy Grade and Quality of Life among Type II Diabetic Patients. Tanta Scientific Nursing Journal. 2021 Nov 1;23(4):268-294.
11. SOLIMAN D, IBRAHIM D, ABASS M. Changes in Kidney Function (GFR), Albuminuria, Electrolytes, and Heart Affection in Diabetic Chronic Kidney Disease Patients. The Medical Journal of Cairo University. 2021 Dec 1;89:2923-2933.
12. Thi TND, Gia BN, Thi HLL, Thi TNC, Thanh HP. Evaluation of urinary L-FABP as an early marker for diabetic nephropathy in type 2 diabetic patients. J Med Biochem. 2020;39(2):224-230.
13. Abdelwahid HA, Dahlan HM, Mojemamy GM, Darraj GH. Predictors of microalbuminuria and its relationship with glycemic control among Type 2 diabetic patients of Jazan Armed Forces Hospital, southwestern Saudi Arabia. BMC Endocr Disord. 2022;22(1):307.
14. Aly M, El-Antouny N, Amr G, Shaker G. Urinary se-cadherin and plasma cystatin C as novel biomarkers of diabetic nephropathy. Zagazig University Medical Journal. 2014 Mar 1;20(2):1-6.