# Urinary levels of endothelin-1 in type 2 diabetes mellitus patients: a non-defined marker of early renal damage

**Key words:** Urinary endothelin-1, diabetes mellitus, renal damage.

**Palabras clave:** Endotelina-1 urinaria, diabetes mellitus, daño renal.

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## Abstract

**Background/Aims:** As urinary endothelin-1 excretion (UET-1) is greater in patients with any cause of renal disease when compared to normal subjects, it eventually may be considered as a renal disease marker. **Methods:** We compared the UET-1 excretion in 67 type 2 diabetes mellitus patients (DM2) with and without hypertension (HT), and with and without microalbuminuria (MA). And in 14 healthy subjects as a control. MA and UET-1 were assessed in 24 h urine, and the difference between groups was tested by ANOVA. The association between UET-1 and variables like evolution time, metabolic control, and renal disease was tested by a multiple correlation test. **Results:** UET-1 was greater in the group of diabetics without MA when compared to the group of hypertensive diabetics with MA ($p < 0.04$). UET-1 was different between the control group and the group of diabetics without HT. **Conclusion:** In our study, the ET-1 excretion tends to be greater in diabetic patients without hypertension as compared with diabetic hypertensive patients. We think the role played by the urinary ET-1 excretion on renal pathophysiology should be clarified in glomerular and proximal tubule pathology.

## Resumen

**Antecedentes/Objetivo:** La excreción de endotelina-1 urinaria (UET-1) es mayor en pacientes con enfermedad renal de cualquier etiología, comparados con sujetos normales, por lo que ésta podría servir potencialmente como un marcador de daño renal. **Metodología:** Comparamos la excreción de UET-1 en 67 pacientes con diabetes tipo 2 (DM2) con y sin hipertensión (HTA), y en presencia o ausencia de microalbuminuria (MA). Estudiamos 14 sujetos sanos como grupo control. La microalbúmina y la endotelina fueron medidas en orina de 24 horas. La diferencia entre los grupos fue determinada mediante prueba ANOVA. Utilizamos una prueba de correlación múltiple para buscar asociación entre la UET-1 y el tiempo de evolución, control metabólico y daño renal. **Resultados:** La excreción de UET-1 fue mayor en el grupo de diabetes sin MA con respecto al grupo de diabetes con MA ($p < 0.04$). Se encontró diferencia significativa entre la excreción de UET-1 del grupo control y el grupo de diabetes sin HTA. **Conclusiones:** En nuestro estudio, la excreción de UET-1 tiende a ser mayor en pacientes diabéticos sin HTA al compararlos con pacientes diabéticos con hipertensión. Consideramos que es necesario continuar estudiando la participación de la UET-1 en la patología renal con modelos que además de evaluar la función glomerular incluyan otros sitios como el túbulo proximal.

## Introduction

Diabetic nephropathy is the main complication in type 2 diabetes mellitus patients (DM2).[1,2] Some hemodynamic factors like arterial hypertension (HT), glomerular hypertension and glomerular hyperfiltration may increase the urinary excretion of albumin and enhance the development of the diabetic nephropathy.[3,4] Endothelin-1 (ET-1) shows a wide spectrum of biological activities such as vasoconstriction, mithogenesis, inhibition of the renal Na/Kasa, vasopressin stimulation and regulation of the atrial natriuretic factor liberation.[5]

The vasoconstrictor effect of ET-1 is greater than that of angiotensin II, renal vessels being ten times more sensible than the rest of the systemic vessels.[6] When administered *in vivo* ET-1 induces an increment in systemic blood pressure which is associated to an increase in the afferent and efferent renal arteriolar resistance, mesangial contraction and proliferation, and with a reduction of the glomerular plasma flow and of the ultrafiltration coefficient,[7,8] that determine a 30 to 50% fall in the single nephron glomerular filtration rate.[9,10]

There are some experimental and clinical data involving ET-1 in chronic renal disease. Experimental data support the role of ET-1 in maintaining high blood pressure in spontaneously hypertensive rats as well as in rats treated with deoxicorticosterone and salt,[11-15] and show that renal dysfunction is reduced by inhibiting the endothelin receptors in experimentally produced diabetic animals. Clinical data have shown that plasma ET-1 is increased in diabetic patients,[16-17] although a recent study failed to demonstrate any relationship between ET-1 plasma concentration and blood pressure levels in type 2 diabetes patients.[18]

The urinary excretion of ET-1 (UET-1) is greater in patients with renal disease of any etiology compared to normal subjects;[16,19] however, UET-1 has not been studied in type 2 diabetes patients with early renal disease and normal or high blood pressure. For this reason, we think that if UET-1 is shown to be related with the presence of microalbuminuria in type 2 hypertensive diabetes patients, it might be used as a marker of renal disease.

In this work we compared the UET-1 in type 2 diabetes mellitus patients with and without high blood pressure and with and without microalbuminuria.

## Material and methods

We did a cross sectional, comparative study in a total of 67 type 2 diabetes mellitus patients from March 2003 to February 2005 at the Instituto de Investigaciones Médicas, University of Guanajuato.

Diabetic patients were grouped according to the presence or absence of both arterial hypertension (HT) and microalbuminuria (MA). Group 1 was formed with eleven hypertensive diabetic patients with MA, group 2 consisted in 22 hypertensive diabetic patients without MA, group 3 included 11 diabetic patients with MA but no HT, and group 4 had 23 diabetic patients without HT or MA. A control group was formed with 14 healthy subjects in order to obtain the reference values for the UET-1.

We included subjects either sex, 36 to 67 years old, with no smoking habits, no history of anticonceptive use in the last two years, no renal, or cardiological diseases, no secondary hypertension, hematuria, systemic or local infectious diseases. Blood pressure readings had to be no greater than 179 over 109 mmHg.[20] High blood pressure was defined by a reading of 140/90 or greater.

A complete clinical history was done in every subject. Blood pressure was determined as the media of three readings obtained in the supine and prone position, with a mercury sphygmomanometer and an appropriate cuff at the dominant arm. Height (shoeless) and weight (with clothes but shoeless) were obtained at first visit. Body mass index was calculated by the Quetelet formula.

Patients who accepted inclusion and were on blood pressure medication were advised to stop treatment during 15 days and their blood pressure was strictly followed for this time. After this period 24 h urine was collected, and after 12 h fasting, 15 mL of morning venous blood were drawn from every subject.

Glycated hemoglobin (HbA1c) was assessed by chromatography or cationic interchange (Biosystems, Barcelona, Spain). Creatinine assay was done by the Jaffé reaction (Spinreact, Spain), Triglycerides, total cholesterol and its fractions HDL, LDL, and VLDL were measured by an enzymatic colorimetric method (Spinreact Spain). Serum insulin was tested by a commercial method based on radioimmunoassay (EURO/DPC Ltd. KHAD1, England) with an intraessay variation coefficient of 10.5%. Creatinine and albumin were determined on the collected 24 h urine. Samples of this urine were freezed for posterior determination of albuminuria (EURO/DPC Ltd. KHAD1), and ET-1 by radioimmunoanalysis using a highly sensible commercial method (Amersham Biosciences code RPA 545). Urinary ET-1 was measured with an intraessay variation coefficient of 7.45%. The assay is based on the competition between unlabelled ET-1 and a fixed quantity of [125I]-labelled ET-1 (synthetic) for a limited number of binding sites on an ET-1 specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added nonradioactive ligand. The antibody bound ET-1 is then reacted with the Amerlex-M second antibody reagent which contains second antibody that is bound to magnetizable polymer particles. Separation of the antibody bound fraction is effected by either magnetic separation or centrifugation of the Amerlex-M suspension and decantation of the supernatant.

Measurement of the radioactivity in the pellet enables the amount of labelled ET-1 in the bound fraction to be calculated. The concentration of unlabelled ET-1 in the sample is then determined by interpolation from a standard curve.

Glomerular filtration rate (GFR) was calculated according to the formula of the MDRD study: $GFR = 186.3 \times (Cr_s)^{-1.154} \times age^{-0.203} \times (0.742$ in women).[21] MA was defined as the presence of 20 to 200 μg/min[22] and insulin resistance was calculated by the homeostatic model.[23]

## Statistics

Mean and standard deviation were obtained for normal variables. Median with the 25 - 75 quartiles for variables with non normal distribution. The 4 groups were compared by ANOVA. An additional comparison of the urinary ET-1 was made between the patient groups and the control group. Kruskal Wallis test was used to analyze MA. Difference between groups was analyzed by the LSD post hoc test. A multiple regression test was done taking as dependent variable urinary ET-1 and as regressor candidates the levels of HbA1c, glycemia, serum insulin and time of duration of the disease. We tested the effect of the urinary ET-1 increase on the risk of developing MA by means of a logistic regression model. Statistical difference was defined by $p < 0.05$.

### Ethical considerations

This investigation was approved by the Institutional Ethics Committee. All subjects signed an informed consent.

## Results

Clinical and biochemical characteristics of the diabetic patients are found in *table I*. Age, BMI, HbA1c, total cholesterol and triglycerides were similar in all groups. Blood pressure values were similar in the 2 groups of hypertensive diabetic patients. The levels of insulin were significantly

**Table I.** Clinical and Biochemical Characteristics of the Diabetic Groups.

| | Group 1 (n = 11) DM+HT+MA | Group 2 (n = 22) DM+HT without MA | Group 3 (n = 11) DM+MA without HT | Group 4 (n = 23) DM without MA neither HT | F | p |
|---|---|---|---|---|---|---|
| Age (years) | 53.8 ± 5.8 | 51.6 ± 8.5 | 51 ± 7.3 | 51.2 ± 7.4 | 0.35 | NS |
| Years of DM | 10 (2-17) | 8 (3-12) | 3 (1-8) | 5(2-10) | NS | |
| MBP (mmHg) | 107 ± 11 | 103 ± 9 | 88 ± 6 | 90 ± 8 | 15.50 | < 0.001 |
| BMI (Kg/M$^2$) | 29.3 ± 4.1 | 29.9 ± 4.9 | 30 ± 5.9 | 28.8 ± 4.5 | 0.23 | NS |
| GFR (mL/min) | 106 ± 30 | 110 ± 45 | 120 ± 42 | 143 ± 33 | 3.61 | < 0.02 |
| MA ($\mu$g/min) | 34 (27-49) | 4.1(2.9-7.3) | 40.5(26.7-90.1) | 2.3 (3-7.6) | 23.86 | < 0.001 |
| HbAC-1 (%) | 9.4 ± 2.3 | 9.3 ± 2.3 | 9.8 ± 3.3 | 10.8 ± 5.6 | 0.66 | NS |
| Glucose (mg/dL) | 143.8 ± 45.7 | 167.3 ± 57.8 | 135.8 ± 63.4 | 142.5 ± 84.4 | 0.76 | NS |
| T. Chol (mg/dL) | 199 ± 25 | 195 ± 49 | 196 ± 45 | 201 ± 41 | 0.09 | NS |
| Triglyc (mg/dL) | 163 ± 91 | 214 ± 174 | 230 ± 121 | 99 ± 108 | 0.52 | NS |
| HDL (mg/dL) | 38 ± 12 | 38 ± 9 | 35 ± 7 | 34 ± 8 | 0.71 | NS |
| LDL (mg/dL) | 128 ± 24 | 114 ± 36 | 123 ± 42 | 123 ± 45 | 0.38 | NS |
| Insulin ($\mu$UI/mL) | 17.9 ± 9 | 12.6 ± 9.2 | 11.1 ± 6.7 | 11 ± 7.2 | 1.90 | NS |
| HOMA | 6.1 ± 3.2 | 5.2 ± 4.2 | 3.6 ± 2.5 | 3.2 ± 1.9 | 3.00 | 0.03 |
| UET-1 (pg/min) | 12.2 ± 4.5 | 15.7 ± 7.9 | 15.7 ± 7 | 18.6 ± 9.5 | 1.61 | NS |

Mean ± SD. Median (IC 95%). MBP = Mean blood pressure, MA = Microalbuminuria, T Chol = total cholesterol, HOMA = insulin resistance index. UET-1 Urinary endothelin-1. DM+HT+MA = Diabetes with hypertension and with microalbuminuria, DM+HT without MA = Diabetes with hypertension without microalbuminuria, DM+MA = Diabetes with microalbuminuria, without hypertension. DM without MA neither HT = Diabetes mellitus without microalbuminuria and without hypertension. Post hoc (LSD) GFR 1 *vs* GFR 4: $p < 0.02$, GFR 2 vs GFR 4: $p < 0.02$, MA 1 *vs* MA 2: $p < 0.00004$, MA 1 *vs* MA 3: $p < 0.02$, MA 1 *vs* MA 4: $p < 0.00004$, MA 2 *vs* MA 3: $p < 0.00001$. MA 3 *vs* Ma 4: $p < 0.00001$. Insulin 1 *vs* Insulin 4: $p < 0.02$ Insulin 1 *vs* Insulin 3: $p = 0.05$, . HOMA 1 *vs* HOMA 4: $p < 0.01$. HOMA 2 *vs* HOMA 4: $p < 0.03$. UET-1 1 *vs* UET-1 4: $p < 0$



greater in the group of the diabetic hypertensive patients with MA, as compared with the group of diabetic patients with microalbuminuria, and without HT, and with the group of diabetic patients without MA and without HT. The levels of the insulin resistance index were significantly lower in the group of diabetic patients without MA and without HT as compared with the groups of diabetic patients with hypertension.

Calculated GFR was greater in the group of diabetics without MA or HT as compared with the two groups of hypertensive diabetic patients ($p < 0.02$). The two groups of non hypertensive diabetic patients had similar values of calculated GFR.

*Figure 1* shows that urinary ET-1 was greater in the group of normotensive diabetic patients without MA as compared with the group of diabetic hypertensive patients with MA (18.6 ± 9.5 pg/min *vs* 12.2 ± 4.5 pg/min, $p = 0.02$), and with the control group (18.6 ± 9.5 pg/min *vs* 11.2 ± 5.3pg/min). *Figure 2* shows that there was a significant difference in the urinary ET-1 excretion of the control group (mean 11.2 ± 5.4) as compared with the non hypertensive diabetic group (mean 17.6 ± 8.7) but there was no difference with the diabetic hypertensive group (mean 14.5 ± 7.1).

Multiple regression analysis was unable to show any correlation among the urinary ET-1 excretion and the levels of HbA1c, glycaemia, serum insulin and time of diagnosis of the disease.

The logistic regression analysis shows that the urinary ET-1 excretion does not represent a risk factor to develop MA.

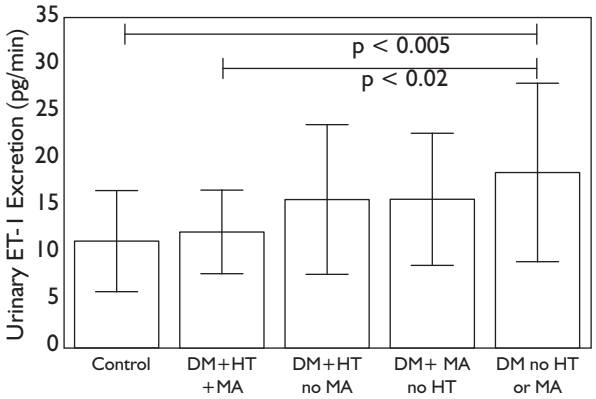


**Figure 1.** Urinary ET-1 excretion on all the groups of study.

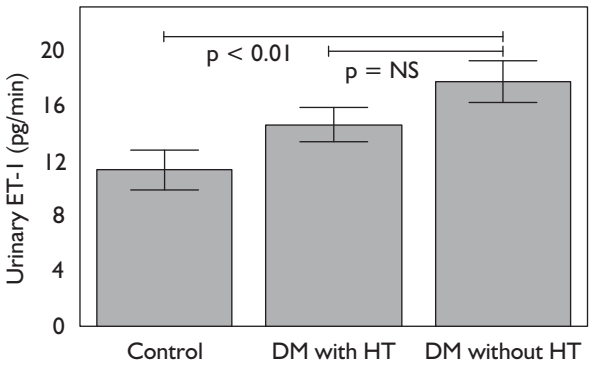


**Figure 2.** Urinary ET-1 excretion on controls, diabetics with hypertension and diabetics without hypertension.

## Discussion

Consistent with the data reported by Shin et al,[24] we found in our study that the diabetic patients with no hypertension or microalbuminuria had greater urinary ET-1 excretion than healthy subjects. The reduced urinary ET-1 excretion suggests that ET-1 synthesis is reduced or its catabolism is enhanced.[25-26] Also, our group of diabetic patients with no hypertension or microalbuminuria had the greater GFR. This may be explained by the diuretic effect of the ET-1 on the tubules, as described by Lariviere[27] and Ohta,[16] who associate the urinary ET-1 excretion levels with the excretion of the N-Acetyl-$^{2}$-D-glucosaminidase (NAG), which is a marker of proximal tubular damage.[27] This association of urinary ET-1 and NAG may support the possibility that the elevated urinary ET-1 excretion seen in some nephropathies is the result of proximal tubular damage, and not of glomerular damage.

The lack of association between the urinary ET-1 excretion and the presence of microalbuminuria found in our study, is consistent with the finding of De Mattia.[28]

On the other hand in the study done by Lee et al, the urinary ET-1 excretion levels were greater in diabetic patients with albuminuria as compared with a control group.[29]

However, this finding might be explained by the inclusion of patients with albuminuria over 200 ¼g/min, while in our study we included only patients with microalbuminuria, in order to study exclusively incipient renal damage.

The lack of association between the urinary ET-1 excretion levels and glycaemia in our study may be explained because our patients were in a better metabolic control, as measured by the HbA1c than the patients studied by Shin et al, in whom they found a positive association between hyperglycemia and urinary ET-1 excretion.[24]

There was no association between the evolution time of the diabetes or the high blood pressure. This finding may be due to the fact that the urinary excretion of ET-1 depends more on the metabolic control than in the time of evolution of the disease.[30]

In conclusion, our study shows that the urinary excretion of ET-1 tends to be greater in the diabetic patients without hypertension as compared with the diabetic hypertensive patients. On the other hand the diabetes evolution time and the hypertension evolution time did not correlate with the urinary ET-1 excretion.

There is no correlation of urinary ET-1 excretion and the mean blood pressure, the metabolic control as measured by the HB1Ac levels, the insulin levels and the GFR.

As clinical studies on the urinary ET-1 excretion are still few, we think it is worth to continue trying to clarify the role played by the urinary ET-excretion on the renal pathophysiology, including models evaluating the proximal tubular and glomerular pathology.

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