

# Urine Transferrin as an Early Endothelial Dysfunction Marker in Type 2 Diabetic Patients Without Nephropathy: A Case Control Study.

**José Juan Sánchez-Hidalgo**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**Juan Antonio SUÁREZ-CUENCA** (✉ [suarej05@gmail.com](mailto:suarej05@gmail.com))

Centro Medico Nacional 20 de Noviembre <https://orcid.org/0000-0002-2098-5658>

**José Juan Lozano-Nuevo**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**Víctor Hugo García-López**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**María Graciela Leal-Gutierrez**

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran: Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran

**Sein Antonio León-Angel**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**María Leslye Ramírez-Villa**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**Martha Elena Rodea-Rubio**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**José Enrique González-Hernández**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**José Antonio Canela-Mayoral**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**Eduardo Murillo-Heredia**

Secretaría de Salud de Mexico: Secretaria de Salud de Mexico

**Eduardo Vera-Gómez**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

**Alejandro Hernández-Patricio**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

**Carlos Ramiro Zamora-Alemán**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

**Gabriela Alexandra Domínguez-Pérez**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

**Juan Ariel Gutiérrez-Buendia**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

**Paul Mondragón-Terán**

Centro Médico Nacional 20 de Noviembre: Centro Medico Nacional 20 de Noviembre

---

## Research

**Keywords:** Urinary transferrin, Chronic kidney disease, type 2 Diabetes, carotid intima-media thickness.

**Posted Date:** May 27th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-477289/v2>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

## Background

Albumin, along with other proteins, is abnormally eliminated via the urine during early stages of diabetic nephropathy. Moreover, endothelial dysfunction (ED) accompanying early diabetic nephropathy may develop even before microalbuminuria is detectable. Transferrin has a molecular weight comparable to albumin, and transferrinuria and microalbuminuria in a 24-hour urine sample may comparably reflect early diabetic nephropathy. However, transferrin physiochemical properties may be related with ED, but these have not been elucidated yet. This case-control study was aimed to evaluate relation between ED and urinary transferrin concentration before early diabetic nephropathy is present.

## Methods

Patients were enrolled from two study sites in Mexico City: Ticoman General Hospital to evaluate control patients; and Dr. Manuel Gonzalez Rivera Specialized Clinic for the Management of the Diabetic Patient for case patients. All patients provided written informed consent. The primary endpoint was the correlation between urinary transferrin concentration and endothelial dysfunction measured in type 2 diabetic patients without albuminuria. ED was evaluated by ultrasonographic validated measurements, which included carotid intima-media thickness (CIMT) and flow mediated dilation (FMD). A power calculation, to detect a statistical difference, with a p value of 0.05, mandated a sample of 60 patients. The patients were tested for serum biomarkers such as glycated hemoglobin, creatinine, cholesterol, triglycerides, and urinary dipstick for albuminuria and urinary tract infection; using inclusion, exclusion and elimination criteria as described.

## Results

The group with type 2 diabetes was older and showed higher serum transferrin and lower urinary transferrin values. Likewise, the group with type 2 diabetes showed subclinical atherogenic risk characterized by lower FMD and higher CIMT and ABI values. Risk factors associated to endothelial dysfunction measured by CIMT were time from diagnosis of diabetes, insulin resistance, dyslipidemia, and male sex. Hba1c and time since diabetes diagnosis correlated both for risk associated to FMD and CIMT. CIMT was the only factor correlated with urinary transferrin values.

## Conclusion

Urinary transferrin correlated to subclinical endothelial dysfunction measured by CIMT in type 2 diabetic patients without nephropathy and can be used to test for early nephropathy in patients without albuminuria.

# Background

Chronic kidney disease (CKD) is a syndrome defined by the persistent alterations in the kidney structure and/or function, which denotes clinical implications. One of its causes is well known to be associated with diabetes mellitus, and identification of early renal damage may help to evaluate the efficacy of different interventions allowing delay of development of diabetic nephropathy and subsequently CKD <sup>1</sup> in subjects at risk.

Microalbuminuria is recognized as valuable marker of incipient diabetic nephropathy. Early diabetic nephropathy is characterised by a pathological increase in glomerular permeability, such that albumin, and other plasma proteins like transferrin, leak from the plasma into the urine. Transferrin's molecular weight is comparable to that of albumin, but it has a higher isoelectric point <sup>2</sup>. Moreover, Masao *et al* found a positive correlation between tubular dysfunction, as evaluated by  $\alpha$ 1 microglobulin,  $\beta$ 2 microglobulin and N-acetyl-D-glycosaminidase, with the degree of microalbuminuria and transferrinuria in patients with diabetic nephropathy <sup>3</sup>. As a matter of fact, the relationship of microalbuminuria and transferrinuria with early tubular damage was further demonstrated quantitatively in biopsy proven diabetic nephropathy <sup>4</sup>.

Endothelial dysfunction (ED) and increased cardiovascular risk has been intimately related with early stages of CKD. It has been found that microalbuminuria is associated with ED, although approximately 20% of patients will develop ED and CKD before microalbuminuria is present <sup>5</sup>. There are few studies showing the relationship between microalbuminuria and indicators of ED with subclinical atherogenesis, whereas the relation among transferrinuria and ED is less clear and has not been explored in patients with diabetes mellitus with very early stages of CKD <sup>6</sup>.

One way to explore this relationship between ED and urinary transferrin in t2DM patients is possible using imaging techniques that can be used as a surrogate marker of vascular dysfunction like flow mediated dilation (FMD) and imaging techniques evaluating atherosclerosis as the carotid intima-media thickness (CIMT) measurement <sup>7</sup>. These tests have shown adequate cardiovascular risk stratification, primarily on events such as infarction, use of lipid lowering therapy and assessment of drug efficacy; yet none of them have been evaluated in patients with renal disease, nor in diabetic patients at risk of diabetic nephropathy <sup>8</sup>.

Thus, we conducted a case-control study to evaluate the relation between transferrinuria with early ED, and indicators of subclinical atherogenesis in patients with type 2 diabetes mellitus before the presence of microalbuminuria and the decline in glomerular filtration rate.

## Methods

### Aim, Design and Setting

The aim of this study was to evaluate the correlation between ED and urine transferrin concentration in patients without diabetic nephropathy. For this purpose, a case-control study was designed and conducted at two sites in Mexico City: Ticomán General Hospital and “Dr. Manuel Gonzalez Rivera” Specialized Clinic for the Management of the Diabetic Patient.

## Study population

The case group involved patients diagnosed with type 2 diabetes mellitus, and the control group was constituted by non-diabetic patients (HbA1c < 6.5%). Patients were excluded if they had evidence of nephropathy, (according to serum creatinine measurement and/or microalbuminuria), evidence of leucocytes in urinary sample, or clinical-biochemical data of urinary tract infection. All patients provided written informed consent.

## Measurements and endpoints:

The primary endpoint was the correlation between transferrinuria and endothelial lesion measured in patients without albuminuria. ED was evaluated by ultrasonographic validated measurements, which included carotid intima-media thickness (CIMT) and flow mediated dilation (FMD). For each group we assigned a total of 30 patients, previously adjusted with the calculated  $n$  for statistical significance. For the patients in each group, we measured serum biomarkers such as glycated hemoglobin, creatinine, cholesterol, triglycerides. All the patients were tested with urinary dipstick for albuminuria and urinary tract infection, using inclusion, exclusion and elimination criteria as described (the full description of these criteria, as well as the ultrasonographic measurements can be found on the supplementary material).

We performed the statistical analysis using IBM SPSS *Statistics* statistical package version 25.1 as well as GraphPad Prism 8.2.1 (441). We tested distributions for normality using the Shapiro Wilk or Kolmogorov Smirnov test depending on the variable type so we could apply measures of central tendency on population variables, as well as to determine median or percentage, depending on the normality testing. We compared categorical variables using contingency tables, using Fisher’s exact test for the association between risk factors and ultrasonographic measurements for endothelial dysfunction, as well as the value of odds ratio, with confidence intervals to 95%. We used of the Pearson correlation factor for the values of endothelial dysfunction and transferrinuria using a two-tailed  $p$  value for statistical significance  $p < 0.05$ .

## Results

A total number of 60 patients with t2DM ( $n = 30$ ) or without t2DM ( $n = 30$ ), all negative for CKD, constituted the study population, whose baseline characteristics are shown in Table 1.

Table 1  
Baseline characteristics of the study population

	t2DM (n = 30)	non-t2DM (n = 30)	p-value
Age (y-o)	56.7 (49.2, 64.2)	42.2 (30.7, 50.5)	<b>0.01</b>
Age > 65y	6 (20.0%)	2 (6.7%)	<b>0.01</b>
Male	15 (50.0%)	10 (33.3%)	0.1
Weight (kg)	67.8 (54.3, 77.1)	70.5 (58.7, 84.2)	0.59
Height (cm)	158.0 (150.0, 168.2)	161.6 (153.0, 168.5)	0.37
BMI (kg/m <sup>2</sup> )	26.7 (23.7, 28.5)	26.7 (23.3, 29.0)	0.9
Comorbidities			
Hypertension	0 (0)	2 (6.7%)	0.39
Dyslipidemia	11 (36.7%)	16 (53.3%)	0.20
Blood Chemistry			
Cholesterol (mg/dL)	175.8 (150.5, 204.7)	175.1 (141.7, 203.7)	0.9
Triglycerides (mg/dL)	146.9 (94.0, 153.0)	147.2 (94.0, 189.0)	0.9
Hb <sub>A1c</sub> (%)	8.3 (6.4, 9.6)	N. A.	<b>0.01</b>
Smoking history	13 (43.3%)	10 (33.3%)	0.41
Time from t2DM onset (years)	10.9 (5.0, 15.0)	N. A.	<b>0.01</b>
T2DM related vascular complications.		N. A.	<b>0.01</b>
None	23 (76.7%)		
Retinopathy	1 (3.3%)		
Neuropathy	2 (6.7%)		
Combined	4 (13.3%)		

	t2DM (n = 30)	non-t2DM (n = 30)	p-value
T2DM drug therapy		N. A.	<b>0.01</b>
MET	3 (10.0%)		
Insulin	2 (6.7%)		
Insulin + MET	9 (30.0%)		
Insulin + MET + Other*	7 (23.3%)		
MET + Other*	7 (23.3%)		
Insulin + Other*	2 (6.7%)		
Renal function			
Serum creatinine	0.76 (0.67, 0.83)	0.75 (0.67, 0.81)	0.76
GFR (mL/min)	99.3 (74.6, 120.1)	118.06 (84.75, 146.65)	0.06
Transferrinuria (mg/ml)	15.3 (0.3–29.7)	19.3 (18.8–12.4)	0.04
Transferrinemia (mg/ml)	2.7 (0.16–0.165)	2.3 (0.3–2.2)	0.27
Transferrinuria/Transferrinemia Index	74.17 (0.00, 126.41)	49.52 (5.09, 68.7)	<b>0.01</b>
Endothelial Dysfunction & Atherogenesis	9.9 (3.2, 16.0)	19.5 (10.9, 26.2)	<b>0.01</b>
FMD value (%)			
CIMT value (mm)	1.19 (0.9, 1.3)	0.66 (0.5, 0.8)	<b>0.01</b>
ABI value	1.25 (1.14, 1.35)	1.19 (1.12, 1.26)	0.14
<p>Values shown were tested for parametric distribution. Data is described as number and percentage as follows n(%), as well as median and interquartile range n(q25, q75) depending on the distribution of variables. BMI=Body mass index. MET=Metformin. FMD=Flow mediated dilation measured by ultrasound. CIMT= Carotid medial thickness measured by ultrasound. ABI= Ankle brachial index. T2DM= Type 2 diabetes. GFR= Glomerular filtration rate measured by Cockcroft-Gault equation. *The drug therapy included under the classification of "other" includes SLGT-2 and DPP-4 Inhibitors.</p> <p>Values highlighted in bold represent statistical significance</p>			

In comparison with non-DM controls, the group with t2DM were older and characterized by higher values of HbA1c, heterogenic microvascular complications and therapy for type 2 diabetes, as well as lower urine transferrin values. Likewise, patients with t2DM showed higher subclinical atherogenic risk characterized by higher CMT values, and lower FMD measurements (Tables 2 and 3).

Table 2  
Risk factors associated with endothelial dysfunction (FMD < 11%)

	<b>n (%)</b>	<b>OR (CI 95)</b>	<b>p-value</b>
Age > 65			
<i>All</i>	5 (8.3%)	1.7 (0.4–7.4)	0.42
<i>Type 2DM</i>	4 (13.3%)	0.7 (0.12–3.99)	0.69
<i>Non-DM</i>	1 (3.3%)	3.6 (0.19–67.6)	0.38
Sex male			
<i>All</i>	12 (20%)	1.3 (0.4–3.9)	0.53
<i>Type 2DM</i>	10 (33.3%)	1.3 (0.3–5.91)	0.7
<i>Non-DM</i>	2 (6.6%)	0.7 (0.11–4.7)	0.7
Smoking			
<i>All</i>	13 (21.6%)	2.4 (0.8–6.9)	0.11
<i>Type 2DM</i>	10 (33.3%)	2.9 (0.59–14.72)	0.18
<i>Non-DM</i>	3 (10%)	1.7 (0.3–9.7)	0.54
Hypertension			
<i>All</i>	1 (1.6%)	1.3 (0.07–22.1)	0.84
<i>Type 2DM</i>	0	N.A.	–
<i>Non-DM</i>	1 (3.3%)	3.6 (0.19–67.6)	0.38
Dyslipidemia (Frederickson type 3)			
<i>All</i>	3 (5%)	0.4 (0.1–1.7)	0.24
<i>Type 2DM</i>	2 (6.6%)	0.5 (0.06–4.4)	0.55
<i>Non-DM</i>	1 (3.3%)	0.4 (0.04–4.7)	0.52
Dyslipidemia (Frederickson type 4)			
<i>All</i>	4 (6.6%)	1.3 (0.3–6.05)	0.68
<i>Type 2DM</i>	2 (6.6%)	1.1 (0.09–14.6)	0.90
<i>Non-DM</i>	2 (6.6%)	2.6 (0.3–20.5)	0.34

	n (%)	OR (CI 95)	p-value
<b>BMI &gt; 25</b>			
<i>All</i>	18 (30%)	1.3 (0.4–4.1)	0.54
<i>Type 2DM</i>	13 (43.3%)	1.8 (0.3–8.3)	0.44
<i>Non-DM</i>	5 (16.6%)	1.3 (0.2–8.4)	0.76
<b>Time from DM diagnosis &gt; 5 years*</b>			
<i>All</i>	19 (31.6%)	5.6 (1.8–17.4)	<b>0.01</b>
<i>Type 2DM (&gt; 5Y)</i>	14 (46.6%)	0.2 (0.02–2.7)	0.27
<i>Non-DM</i>	0	N. A.	–
<b>Hb<sub>A1c</sub> &gt;8.3%</b>			
<i>All</i>	9 (15%)	3.07 (0.8–10.6)	0.07
<i>Type 2DM</i>	9 (30%)	1.08 (0.2–4.7)	0.90
<i>Non-DM</i>	0	N. A.	–
<b>Transferrinuria</b>			
<i>All</i>	11 (18.3%)	0.73 (0.26–2.05)	0.55
<i>Type 2DM</i>	9 (30%)	1.08 (0.24–4.7)	0.90
<i>Non-DM</i>	2 (6.6%)	0.36 (0.05–2.2)	0.28
<b>Transferrinemia</b>			
<i>All</i>	2 (3.3%)	0.32 (0.06–1.6)	0.18
<i>T2DM</i>	2 (6.6%)	1.1 (0.09–14.6)	0.9
<i>Non-DM</i>	0	N. A.	–
<p>*For the odds ratio calculated in the overall group, the values were taken from the groups as having diabetes or being non-diabetic with the values equals to 0 for the non-diabetic group. For the odds ratio calculated in the group of type 2 DM, the risk was calculated whether they had more than 5 years of being diagnosed with diabetes. OR=Odds ratio. CI=Confidence interval. BMI= Body mass index.</p> <p>Values highlighted in bold represent statistical significance.</p>			

Table 3  
Risk factors associated with endothelial dysfunction (CIMT >0.7)

	N (%)	OR (CI 95)	p-value
Age > 65			
<i>All</i>	6 (10%)	1.2 (0.2–5.7)	0.73
<i>Type 2DM</i>	6 (20%)	0.2 (0.01–5.03)	0.38
<i>Non-DM</i>	0	N. A.	–
Sex male			
<i>All</i>	20 (33.3)	4.2 (1.2–13.8)	<b>0.01</b>
<i>Type 2DM</i>	14 (46.6%)	1 (0.05–17.6)	1.00
<i>Non-DM</i>	6 (20%)	8.5 (1.4–49.5)	<b>0.01</b>
Smoking			
<i>All</i>	14 (23.3%)	0.9 (0.3–2.7)	0.9
<i>Type 2DM</i>	11 (36.6%)	N. A.	–
<i>Non-DM</i>	3 (10%)	1 (0.19–5.2)	1
Hypertension			
<i>All</i>	2 (3.3%)	N. A. (0-INF)	–
<i>Type 2DM</i>	0 ()	N. A.	–
<i>Non-DM</i>	2 (6.6%)	N. A. (0-INF)	–
Dyslipidemia (Frederickson type 3)			
<i>All</i>	6 (10%)	0.69 (0.18–2.6)	0.59
<i>Type 2DM</i>	4 (13.3%)	N. A. (0-INF)	–
<i>Non-DM</i>	2 (6.6%)	0.9 (0.14–5.9)	0.90
Dyslipidemia (Frederickson type 4)			
<i>All</i>	2 (3.3%)	0.16 (0.03–0.88)	<b>0.03</b>
<i>Type 2DM</i>	2 (6.6%)	0.07 (0.003-1.7)	0.1
<i>Non-DM</i>	0	N. A.	–

	N (%)	OR (CI 95)	p-value
BMI > 25			
<i>All</i>	25 (41.6%)	1.3 (0.4–3.9)	0.59
<i>Type 2DM</i>	18 (60%)	1.8 (0.1–31.9)	0.68
<i>Non-DM</i>	7 (23.3%)	2.1 (0.3-13.04)	0.40
Time from DM diagnosis > 5 years*			
<i>All</i>	28 (46.6%)	32.6 (6.3-167.2)	<b>0.001</b>
<i>Type 2DM</i>	22 (73.3%)	N. A.	–
<i>Non-DM</i>	0	N. A.	–
Hb <sub>A1c</sub> >8.3%			
<i>All</i>	12 (20%)	5.04 (1.01–25.09)	<b>0.04</b>
<i>Type 2DM</i>	12 (40%)	N. A.	–
<i>Non-DM</i>	0	N. A.	–
Transferrinuria			
<i>All</i>	16 (26.6%)	0.69 (0.24–1.98)	0.50
<i>Type 2DM</i>	13 (43.3%)	0.86 (0.04–15.2)	0.92
<i>Non- DM</i>	3 (10%)	0.45 (0.08–2.3)	0.34
Transferrinemia			
<i>All</i>	5 (8.3%)	0.74 (0.17–3.1)	0.68
<i>T2DM</i>	3 (10%)	N. A.	–
<i>Non-DM</i>	2 (6.6%)	1.2 (0.17–8.2)	0.84
*For the odds ratio calculated in the overall group, the values were taken from the groups as having diabetes or being non-diabetic with the values equals to 0 for the non-diabetic group. For the odds ratio calculated in the group of type 2 DM, the risk was calculated whether they had more than 5 years of being diagnosed with diabetes. OR=Odds ratio. CI=Confidence interval. BMI= Body mass index.			
Values highlighted in bold represent statistical significance.			

Risk factors associated with atherogenic progression, as evaluated by higher CIMT, were male sex particularly in non-DM subjects, hypertriglyceridemia and HbA1c > 8.3% (cutoff, median value) (Table 3); while time from t2DM diagnosis and HbA1c > 8.3% were associated with endothelial dysfunction, reflected by FMD < 11% cutoff (Table 2).

Furthermore, HbA1c and time from t2DM diagnosis were related with CIMT values ( $r = 0.48$ ,  $p < 0.001$  and  $0.48$ ,  $p < 0.001$ , respectively; Fig. 1B and 1C), whereas urine transferrin was related with CIMT value ( $r =$

0.37,  $p = 0.04$ ), being particularly significant for the t2DM group (Fig. 1D). On the other hand, HbA1c was negatively related with FMD ( $r = -0.26$ ,  $p = 0.04$ ; Fig. 1A).

Based on this observation, we developed a urinary transferrin/serum transferrin index to evaluate and stratify as high-risk patients those with a cutoff value of 74.17 ( $p = 0.01$ ). We could identify patients with a value higher than 74 as having a high risk of developing nephropathy before they can develop microalbuminuria.

## Discussion

The key finding of the present study states that urinary transferrin was significantly related with subclinical atherogenesis, particularly in patients with diagnosis of t2DM, who had not developed renal disease yet. This was primarily evidenced by a positive correlation between urinary transferrin measurement and CIMT value, being particularly significant for subjects with t2DM. Such correlation results in a potentially useful tool for clinical assessment of a very early endothelial damage to the kidney.

Microalbuminuria has been traditionally used for the assessment of early renal damage in patients with T2DM. However, approximately 30 to 45% of t2DM patients may show a diminished glomerular filtration rate, even in the absence of albuminuria. Therefore, clinical characterization of new biomarkers is required<sup>9</sup>. To date, there has not been described the correlation between transferrinuria in a random urinary sample and incipient nephropathy with t2DM as an early endothelial damage biomarker in patients without known renal disease.

Urinary transferrin, as well as several urine biomarkers such as ceruloplasmin, immunoglobulin G, podocalyxin, neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-beta-glycosaminidase,  $\alpha$ -1-microglobulin, 8-hydroxy-deoxyguanosine, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-18 and cystatin C, have shown to reflect early renal damage progression when assessed altogether with microalbuminuria<sup>10</sup>. However, urinary transferrin alone may also correlate with early vascular damage reflected by ED and subclinical atherogenesis, leading to arterioles inability of adaptive vasodilation and to progressive increase of vascular stiffness<sup>11</sup>. This indicates that urinary transferrin is an early marker of vascular disease, occurring even before overt microalbuminuria in patients with t2DM.

While the relation of urinary transferrin with early renal damage and subclinical atherogenesis is not completely clear, possible explanation is acting through different pathological mechanisms. Although the underlying problem often cannot be treated, extensive studies in experimental animals and humans suggest that progressive CKD may be largely due to secondary factors that are sometimes unrelated to the activity of the initial disease<sup>12</sup>. Some of these secondary factors include compensatory response to nephron loss to maintain the total glomerular filtration rate (GFR); direct endothelial cell damage, like that

induced by systemic hypertension; and marked tubulointerstitial injury (tubular dilatation, interstitial fibrosis), even if the primary process is a glomerulopathy <sup>13</sup>.

Zylka et al found the highest relationship between biomarkers associated with glomerular damage (in comparison to albuminuria) and diminished glomerular filtration rate that were evaluated in a population similar to the present study, in which it stands for transferrin, immunoglobulin G, ceruloplasmin, type IV collagen, glycosaminoglycans, prostaglandin D synthase lipocalin type, fibronectin, vascular endothelial growth factor, cystatin C, and nefinina <sup>14</sup>. Besides, Kim et al described a correlation between tubulointerstitial damage in t2DM with nephropathy, including different biomarkers such as urinary transferrin, that could lead to evaluate histopathological damage <sup>15</sup>. Therefore, the correlation of urinary transferrin with ED suggests that glomerular and tubulointerstitial damages are related with vascular dysfunction, and urinary transferrin may be a useful marker of such dysfunction.

In addition, our results suggest that either the urinary transferrin or the urinary/serum transferrin index are useful to identify patients with diabetic nephropathy at early risk for ED and subclinical atherogenesis. These finding suggests the dynamic participation of the whole transferrin metabolism as related with early renal and vascular damages. The clinical usefulness of transferrinuria/transferrinemia index in the evaluation of early renal damage and subclinical atherogenesis, as a part of a routine test to identify high risk population with t2DM, are to be developed, and merits further evaluation by developing the specific concentration that could lead the patient at high risk, develop diabetic nephropathy.

The main limitation of our study was the heterogeneous characteristics between cases and controls within the study population, particularly in terms of age. However, no significant association was found between age and FMD nor CIMT values that could affect the study results. Another limitation was the determination of albuminuria with a bedside urine dipstick test in a urine single sample. Since this method is more sensitive for albumin (even with the false positive, false negative results that can occur), it resulted adequate for our analysis <sup>1</sup>. Finally, we used urine transferrin measured in a single morning urine sample, which did not show significant difference with 24h urine transferrin.

In conclusion, urine transferrin may be related with early endothelial dysfunction and subclinical atherogenesis in patients with t2DM and very early renal damage. This relation may be useful to stratify patients at higher vascular risk as well as early nephropathy, even before microalbuminuria becomes evident.

## Abbreviations

**CKD:** Chronic kidney disease.

**ESRD:** End stage renal disease.

**QOL:** Quality of life.

**ED:** Endothelial dysfunction.

**AKI:** Acute kidney injury.

**CKD-EPI:** Chronic kidney disease epidemiology collaboration.

**CIMT:** Carotid intima media thickness.

**FMD:** Flow mediated dilation.

**T2DM:** type 2 Diabetes mellitus.

**HbA1c:** Glycated hemoglobin.

**BMI:** Body mass index.

**MET:** Metformin.

**GFR:** Glomerular filtration rate.

**ABI:** Ankle brachial index.

**OR:** Odds ratio.

**CI:** Confidence interval.

**DM:** Diabetes mellitus.

**NA:** Not applicable.

## **Declarations**

*Ethics approval and consent to participate.*

The information regarding the safety of this study was collected as stated in the Human Rights Protection Agreement for the Human Dignity applied to Medicine and Biology, to protect the personal information provided by the patients at the time of the interview (according to the European Council 2007) as well as the General Law for Personal Data Protection (according to Mexican Law).

We included the Helsinki declaration as stated by The World Medical Association (WMA) as follows: The WMA has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

The ethics approval committee at Xoco General Hospital acknowledged the protocol with the following registration number: 2007 010 23 20.

We provided a written consent for use and management of personal data to all patients.

#### *Availability of data and materials*

The datasets generated and analyzed during the current study are not publicly available due to privacy policies of the hospital and patient's information; but are available from the corresponding author on reasonable request.

#### *Competing interests*

The authors declare that they have no competing interests.

#### *Funding*

The study was partially funded by the Miguel Aleman Foundation A.C. which principal mission is to encourage, develop, protect, and support, all kind humanistic, scientific, social, artistic, and educative activities to generate knowledge. Its role within this study was to support the acquisition of the transferrin laboratory test to evaluate serum and urine samples as well as to provide basic tools for clinical evaluation of the subjects. There was no conflict regarding the analysis and interpretation of the data in writing the manuscript due to intervention of the Foundation.

#### *Authors' contributions*

SH performed the ultrasound measurements, the clinical evaluation and the patient's interview; likewise, he was responsible for the blood sample collections as well as the sample labeling, handling, and transport to the laboratory for sample processing in both the case and control groups of patients. He was part of the statistical analysis and accountable on the selection of the control cases, providing written consent and information regarding the study protocol at Ticoman General Hospital.

LA and LG managed the sample labeling, handling, and transport to the laboratory for sample processing in both the case and control groups of patients.

GH, CM, and MH were accountable on the selection of the case patients, provided written consent, as well as the information regarding the study protocol at the Specialized Clinic for the Management of the Diabetes Patient.

VG, HP, ZA, DP and GB provided the analysis and interpretation of patients' data concerning the transferrin urine and blood samples at the Clinical Research Laboratory once the samples were delivered. They were associated with the ultrasound training for the accurate measurements of CMT and FMD values.

All authors read and approved the final manuscript.

### *Acknowledgements*

Not applicable

## References

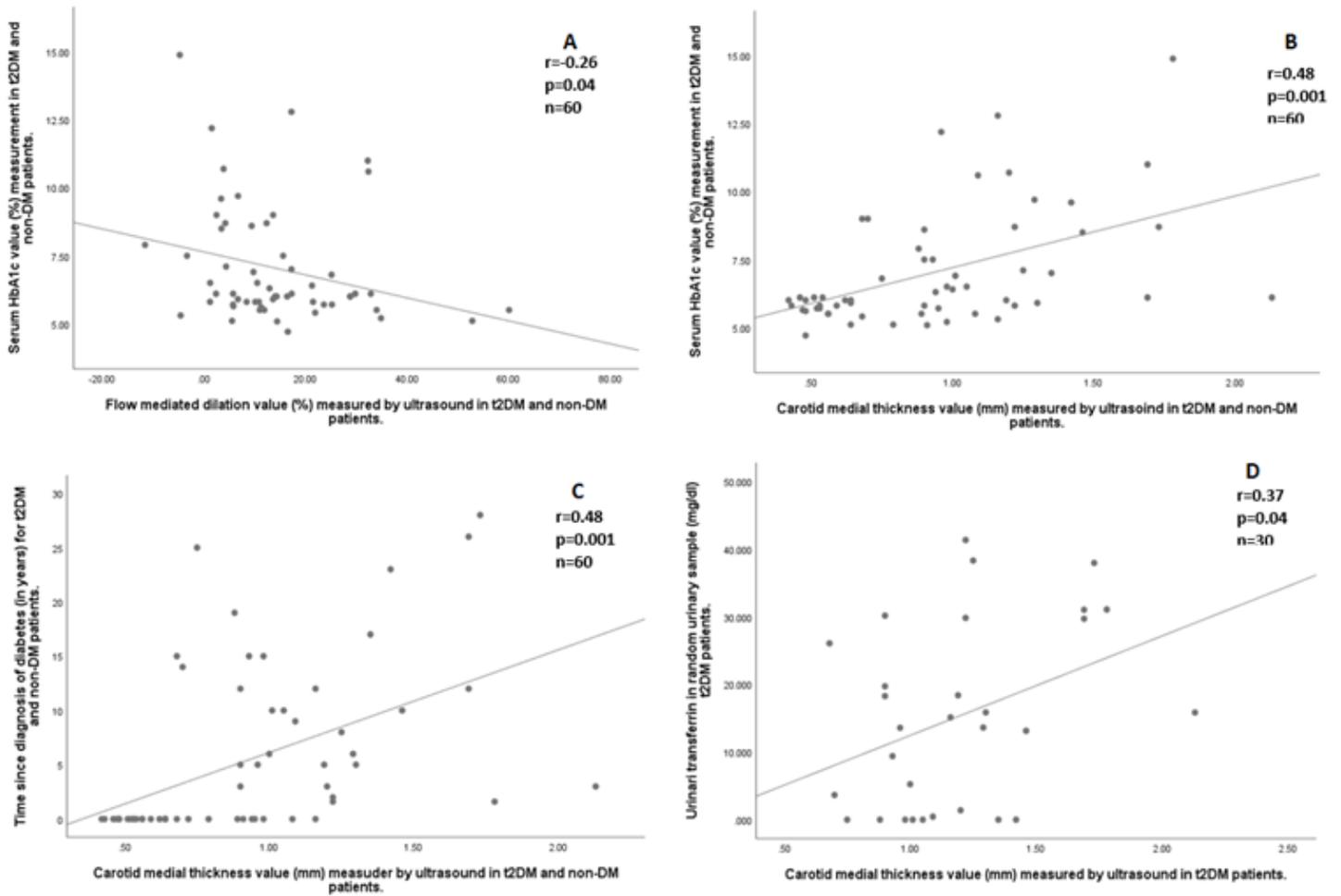
1. Romagnani P, Remuzzi G, Glassock R, Levin A, Jager KJ, Tonelli M, Massy Z, Wanner C, Anders HJ. Chronic kidney disease. *Nat Rev Dis Primers*. 2017 Nov 23;3:17088. doi: 10.1038/nrdp.2017.88.
2. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. *Cell*. 2010 Jul 9;142(1):24–38. doi: 10.1016/j.cell.2010.06.028.
3. Kanauchi M, Nishioka H, Hashimoto T, Dohi K. Diagnostic significance of urinary transferrin in diabetic nephropathy. *Nihon Jinzo Gakkai Shi*. 1995 Nov;37(11):649 – 54.
4. Kanauchi M, Akai Y, Hashimoto T. Transferrinuria in type 2 diabetic patients with early nephropathy and tubulointerstitial injury. *Eur J Intern Med*. 2002 May;13(3):190–3. doi:10.1016/s0953-6205(02)00028-6.
5. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. *Curr Opin Nephrol Hypertens*. 2011 May;20(3):246 – 57. doi: 10.1097/MNH.0b013e3283456546.
6. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging*. 2010 Aug;26(6):631 – 40. doi: 10.1007/s10554-010-9616-1. Epub 2010 Mar 26.
7. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrang D, Lieberman EH, Ganz P, Creager MA, Yeung AC, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995 Nov 1;26(5):1235-41. doi: 10.1016/0735-1097(95)00327-4.
8. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, Cosentino F, Deanfield J, Gallino A, Ikonomidis I, Kremastinos D, Landmesser U, Protogerou A, Stefanadis C, Tousoulis D, Vassalli G, Vink H, Werner N, Wilkinson I, Vlachopoulos C. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil*. 2011 Dec;18(6):775 – 89. doi: 10.1177/1741826711398179.
9. Schwab SJ, Dunn FL, Feinglos MN. Screening for microalbuminuria. A comparison of single sample methods of collection and techniques of albumin analysis. *Diabetes Care*. 1992 Nov;15(11):1581-4. doi: 10.2337/diacare.15.11.1581.
10. Narita T, Hosoba M, Kakei M, Ito S. Increased urinary excretions of immunoglobulin g, ceruloplasmin, and transferrin predict development of microalbuminuria in patients with type 2 diabetes. *Diabetes Care*. 2006 Jan;29(1):142-4. doi: 10.2337/diacare.29.1.142.

11. Widmer RJ, Samuels B, Samady H, Price MJ, Jeremias A, Anderson RD, Jaffer FA, Escaned J, Davies J, Prasad M, Grines C, Lerman A. The functional assessment of patients with non-obstructive coronary artery disease: expert review from an international microcirculation working group. *EuroIntervention*. 2019 Mar 20;14(16):1694–1702. doi: 10.4244/EIJ-D-18-00982.
12. Yu HT. Progression of chronic renal failure. *Arch Intern Med*. 2003 Jun 23;163(12):1417-29. doi: 10.1001/archinte.163.12.1417.
13. Nath KA. Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis*. 1992 Jul;20(1):1–17. doi: 10.1016/s0272-6386(12)80312-x.
14. Żyłka A, Dumnicka P, Kuśnierz-Cabala B, Gala-Błądzińska A, Ceranowicz P, Kucharz J, Ząbek-Adamska A, Maziarz B, Drożdż R, Kuźniewski M. Markers of Glomerular and Tubular Damage in the Early Stage of Kidney Disease in Type 2 Diabetic Patients. *Mediators Inflamm*. 2018 Aug 9;2018:7659243. doi: 10.1155/2018/7659243. PMID: 30158836.
15. Kim SS, Song SH, Kim IJ, Kim WJ, Jeon YK, Kim BH, Kwak IS, Lee EK, Kim YK. Nonalbuminuric proteinuria as a biomarker for tubular damage in early development of nephropathy with type 2 diabetic patients. *Diabetes Metab Res Rev*. 2014 Nov;30(8):736 – 41. doi: 10.1002/dmrr.2546.

## Supplementary File

Supplemental Materials file is not available with this version.

## Figures



**Figure 1**

Correlations between ultrasound measurements, serum, and urinary samples. Panel A shows the correlation between serum HbA1c and FMD values in the total study group. Panel B shows the correlation between serum HbA1c values and CIMT values in the total study group. Panel C shows the correlation between time since diagnosis of diabetes and CIMT value in the total study group. Panel D shows the correlation between transferrinuria in random urine sample in t2DM patients and CIMT values in t2DM patients. At the top right corner of each panel, it is shown the correlation factor ( $r$ ) statistical significance ( $p$ -value), and the total of patients evaluated for each correlation ( $n$ ).