Nephropathy Following Type 2 Diabetes Mellitus in Tunisian Population

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ABSTRACT

Objective: *The purpose was to compare the characteristics of Tunisians with Type 2 diabetes mellitus (Type 2 DM) and nephropathy with those without nephropathy. This study assessed whether or not phenotypic characteristics can predict nephropathy development in Type 2 DM. The prevalence of nephropathy in Tunisian Type 2 DM patients, and their relationship with clinical and biochemical factors as well as chronic complications of the disease were determined.*

Methods: *This was a cross-sectional study of patients with diabetes diagnosed between January 2008 and December 2010. Altogether, 73 Type 2 DM and 42 healthy volunteers from the Basic Health Group of Sousse, were targeted for the study. Clinical, biochemical data, as well as complications of diabetes were collected. Kidney malfunction was defined by glomerular filtration rate (GFR).*

Results: *Diabetic patients were older. Diabetic women were more likely to have higher body mass index than men (*$p = 0.004$*). Obesity was more in women than men (60/23%). Complications including hypertension and dyslipidaemia were co-associated in women. Urinary creatinine clearance in Type 2 DM patients without nephropathy was significantly lower than in healthy participants (*$p < 0.0001$*). Microalbuminuria and urinary creatinine clearance were associated only in women with Type 2 DM with nephropathy (*$R^2 = 0.95$*); 1.5% of Type 2 DM patients without nephropathy had GFR < 60 mL/min/1.73m^2 and 76% had a GFR between 60 and 89 mL/min/1.73m^2. Glomerular filtration rate difference between Type 2 DM patients with/without nephropathy, as well as between Type 2 DM patients with nephropathy/Type 2 DM without nephropathy, and with retinopathy was not significant.*

Conclusions: *By analysing factors associated with nephropathy in Type 2 DM Tunisian patients, this study demonstrated their susceptibility to nephropathy. In addition, retinopathy is potentially associated with incipient nephropathy in Type 2 DM Tunisian patients.*

Keywords: Glomerular filtration rate (GFR), Type 2 diabetes mellitus, nephropathy, retinopathy

Nefropatía tras la Diabetes Mellitus Tipo 2 en la Población Tunesina

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RESUMEN

Objetivo: *El propósito fue comparar las características de los tunesinos con diabetes mellitus tipo 2 (DMT2) y nefropatía, con aquéllos que no padecen nefropatía. Este estudio evaluó la posibilidad de saber si las características fenotípicas pueden predecir el desarrollo de una nefropatía en pacientes de DMT2. Se determinó la prevalencia de la nefropatía en los pacientes tunesinos con DMT2, y su relación con factores clínicos y bioquímicos, así como las complicaciones crónicas de la enfermedad.*

Métodos: *Se realizó un estudio transversal de pacientes con diabetes diagnosticada entre enero de 2008 y diciembre de 2010. En total, 73 voluntarios con DMT2 y 42 saludables del Grupo Básico de Salud de Sousse, fueron escogidos para el estudio. Se recogieron los datos clínicos y bioquímicos, así como las complicaciones por diabetes. El grado de mal funcionamiento renal fue determinado por la tasa de filtrado glomerular (GFR).*

Resultados: *Los pacientes diabéticos tenían más edad. Las mujeres diabéticas presentaban una mayor probabilidad de tener un índice de masa corporal más alto que los hombres (*$p = 0.004$*). Hubo mayor*

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obesidad en las mujeres que en los hombres (60/23%). Las complicaciones – incluyendo hipertensión y dislipidemia – estuvieron co-asociadas en las mujeres. La depuración de la creatinina urinaria en los pacientes de DMT2 sin nefropatía fue significativamente más baja ($p < 0.0001$*) que en los participantes saludables. La microalbuminuria y la depuración de la creatinina urinaria estuvieron asociadas en las mujeres con DMT2 con nefropatía ($R^2 = 0.95$); 1.5% de los pacientes con DMT2 sin nefropatía, tuvo una tasa GFR < 60 mL/min/1.73m^2 y 76% tuvo una GFR entre 60 y 89 mL/min/1.73m^2. La diferencia de la tasa de filtrado glomerular entre los pacientes de DMT2 con/sin nefropatía, así como entre los pacientes de DMT2 con nefropatía/DMT2 sin nefropatía, y con retinopatía, no fue significativa.*
Conclusiones: *Analizando factores asociados con la nefropatía en pacientes tunesinos con DMT2, este estudio demostró que estos últimos son susceptibles a la nefropatía. Además, la retinopatía se halla potencialmente asociada con la nefropatía incipiente en los pacientes tunesinos que padecen DMT2.*

Palabras claves: Tasa de filtrado glomerular (GFR), diabetes mellitus tipo 2 (DMT2), nefropatía, retinopatía

West Indian Med J 2012; 61 (9): 882

INTRODUCTION

Type 2 diabetes mellitus (Type 2 DM) is a common metabolic disease, characterized by glycaemia > 1.26 g/L (7 mmol/L), that could be associated with many co-morbidities including nephropathy (1−2). Type 2 diabetes mellitus affects many ethnic groups in Tunisia (3) and diabetic nephropathy is considered the most frequent cause of end-stage renal disease (ESRD) in Africa and developing countries (4).

Clinical analysis has implicated several different factors in nephropathy (5−6) with a possible genetic predisposition (7). Strong risk factors include sustained hyperglycaemia, hypertension, smoking and obesity. Whereas weak risk factors are dyslipidaemia and physical inactivity. A large body of work characterized nephropathy, a microvascular disease, with a progressive albuminuria, and a decrease of the glomerular filtration rate [GFR] (6, 8). The increased body mass index (BMI) associated with hypertension makes the Type 2 DM population in Tunisia more susceptible to nephropathy. This study focusses on diabetic nephropathy in order to improve Type 2 DM patients' outcome and to avoid significant associated morbidity and mortality. Early detection of nephropathy may prevent end stage kidney disease.

SUBJECTS AND METHODS

This study used data generated from 115 Tunisian adults who visited the Basic Health Group of Sousse over two years (2008–2010). Patients, who gave their consent, were included if they had Type 2 DM (73 patients, sex ratio male/females 0.3) or not (42 healthy volunteers as normal controls, sex ratio male/females 0.3). Type 2 diabetes mellitus was diagnosed after at least two separate overnight fasting venous plasma glucose concentrations above 6.1 mmol/L (9), or above 126 mg/dL by the American Diabetes Association (ADA) criteria (10). Patients were excluded from the study if they had Type 1 DM, or active chronic inflammatory diseases or had been treated with chemotherapy for cancer.

We recorded the main clinical markers in all subjects, co-morbidities (macrovascular and microvascular complications including retinopathy, polyneuritis, nephropathy), family history of diabetes, year of diabetes diagnosis, tobacco consumption, and other pharmacological treatments. Type 2 DM was treated by insulin and/or oral hypoglycaemic agents (10).

Diabetic nephropathy was diagnosed in the presence of microalbuminuria or proteinuria. Diabetic retinopathy was diagnosed by ophthalmological fundoscopic examination recognizing the features of the eye changes in diabetes. Hypertension was considered if the blood pressure was more than 140/90 mmHg according to ADA criteria (11), or if the patient was taking anti-hypertensives.

Blood pressure, weight and height measurements were performed. Systolic and diastolic blood pressures were taken with a standard manual sphygmomanometer. Normal blood pressure was taken as < 130/80 mmHg. The BMI (kg/m^2) was also determined. A BMI of ≥ 30 kg/m^2 was an indicator of obesity.

Standard biological parameters including the haemogram were measured under fasting conditions on the same day of the clinical examination. Venous plasma glucose was measured by the glucose oxidase method with an automated spectrophotometer A25-autoanalyzer (Biosystems). Glycated haemoglobin molecule (HbA_{1c}) measurement (normal range 4−5.5%) was carried out by turbidimetric inhibition immunoassay with the Cobas c 111 analyser (Roche). Serum lipid levels (total cholesterol, triglycerides, high- and low density lipoprotein (HDL, LDL)) were performed by enzymatic methods using the Vitalab Flexor E (Vital Scientific).

Liver enzyme levels including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured for all patients. The following parameters were measured to define renal function: urea, creatinine, microalbuminuria, and creatinine clearance (CC). Microalbuminuria was defined as < 2.8 g/mol for women and < 2.3

g/mol for men (12). Estimated GFR (normal range > 60 mL/min/1.73 m^2), an important marker of renal function, was calculated according to the Modified Diet in Renal Disease (MDRD) formula based on serum creatinine concentration, age, race, and gender (13−16). Glomerular filtration rate stages were classified as previously described (6).

Statistical analysis was performed using the statistical software GraphPad Prism (version 5.01). Descriptive data were analysed using means, standard error of the mean (SE), percentages and variances. Data are expressed as means (SE). Comparison of quantitative variables was performed using the two-tailed Student's *t*-test, whereas comparison of qualitative variables was performed by Chi-square test. Linear regression and Pearson correlation were used to evaluate the associations and the correlation between kidney factors. Statistical significance was inferred at $p < 0.05$.

RESULTS

Participants' characteristics at baseline are shown in Table 1. The study population consisted of 73 Type 2 DM patients. The mean age (SE) was 59.3 (1.1) years without a significant difference between genders (Table 1). The distribution between treatment groups was as follows: one oral hypoglycaemic agent (26.8%), two oral agents (13.4%), three oral agents (1.5%), oral agents and insulin (34.4%) and insulin [23.9%] (Table 2). Table 2 indicates that sulfonylurea was taken alone in 11.9% of Type 2 DM patients, and in combination with other drugs in 40.3% of patients.

Several gender differences were noticed (Table 1). The BMI for women was 31.6 (0.7) kg/m^2 and 26.3 (1.2) kg/m^2 for men. Obesity is estimated at 60% for women and 23% for men explaining the enhanced lipid-lowering agents used by women. Women had significantly higher CC, whereas

Table 1: Baseline characteristics

	Total	Men	Women	*p**
Mean (SE)				
Age (years)	59.3 (1.1)	64.1 (2.6)	58.1 (1.2)	0.666
BMI (kg/m^2)	30.5 (0.7)	26.3 (1.2)	31.6 (0.7)	0.004
Venous plasma glucose (mmol/L)	8.7 (0.4)	9.1 (0.8)	8.7 (0.4)	0.526
HbA_{1c} (%)	7.5 (0.2)	7.6 (0.6)	7.6 (0.3)	0.120
Duration of disease (Years)	10.6 (1)	8.8 (2)	11 (1)	0.289
Systolic blood pressure (mmHg)	136.3 (3.1)	148 (0.8)	132.8 (0.3)	0.285
Diastolic blood pressure (mmHg)	76.8 (1.9)	80.8 (0.4)	77.2 (0.2)	0.095
Triglycerides (mmol/L)	1.5 (0.1)	1.6 (0.1)	1.5 (0.1)	0.268
Total cholesterol (mmol/L)	4.6 (0.1)	4.5 (0.2)	4.8 (0.1)	0.088
High density lipoprotein (HDL, mmol/L)	1.2 (0.1)	1.1 (0.1)	1.2 (0.1)	0.083
Low density lipoprotein (LDL, mmol/L)	2.8 (0.1)	2.7 (0.1)	2.9 (0.1)	0.391
Iron (µmol/L)	15.5 (0.6)	17.4 (2.1)	15.3 (0.6)	0.340
Aspartate aminotransferase (AST, IU/L)	19.5 (0.9)	19.1 (1.3)	19.9 (1)	0.841
Alanine aminotransferase (ALT, IU/L)	27.2 (1.2)	25.8 (1.6)	27.9 (1.5)	0.534
Creatinine (µmol/L)	68.3 (2)	77.2 (2.9)	67 (2.2)	0.051
Urea (mmol/L)	4.8 (0.2)	5.8 (0.4)	4.5 (0.2)	0.0009
Microalbuminuria (g/mol)	6.6 (1.4)	4.5 (0.7)	7.9 (1.7)	0.34
Creatinine clearance (mL/min)	79.3 (5)	82.2 (5.8)	83.3 (4.5)	0.054
White blood cells (10^3/mm^3)	6.8 (0.3)	7.6 (0.6)	6.7 (0.3)	0.326
Haemoglobin (g/dL)	12.3 (0.2)	13.1 (0.4)	12.3 (0.1)	0.049
Platelets (10^3/mm^3)	266.6 (8.8)	247.3 (15)	276 (9.7)	0.718
Per cent patients with feature				
Macrovascular disease	10	14	8	< 0.0001
Retinopathy	27	43	24	0.0070
Polyneuritis	37	29	39	0.1791
Nephropathy	11	14	10	0.5139
Family history	75	57	80	0.0008
Cigarette smoking	8	29	3	< 0.0001
Insulin therapy	53	57	53	0.6698
Sulfonylurea therapy	47	50	46	0.6711
Glinides therapy	1	0	2	0.4773
Biguanides therapy	52	36	56	0.0070
Glitazones therapy	7	7	7	0.7817
ACE inhibitor therapy	14	14	14	0.8385
Anti-hypertensives (Not ACE inhibitor)	41	43	41	0.8861
Lipid-lowering agents	12	0	15	0.0002

* Gender difference, ACE: Angiotensin-converting enzyme. Data are means (SE) or percentage.

Table 2: Type 2 diabetes mellitus patients' hypoglycaemic treatments

	Therapy percentage	Therapy	Patients percentage
Mono-therapy	50.7	Insulin	23.9
		Sulfonylurea	11.9
		Biguanides	14.9
Bi-therapy	25.4	Insulin + biguanides	7.5
		Sulfonylurea + insulin	4.5
		Sulfonylurea + glinides	1.5
		Sulfonylurea + glitazones	1.5
		Sulfonylurea + biguanides	10.4
Tri-therapy	23.9	Insulin + sulfonylurea + biguanides	19.4
		Insulin + sulfonylurea + glitazones	1.5
		Insulin + biguanides + glitazones	1.5
		Sulfonlylurea + biguanides + glinides	1.5

men had significantly higher creatinine, urea levels, and haemoglobin percentage (Table 1). In addition, men had more significant smoking habits than women.

Patients with Type 2 DM were affected by various co-morbidities. Macrovascular disease and retinopathy were significantly enhanced in males compared to women. Women were more likely to have polyneuritis, even though not significant, compared with men. Family history of diabetes was significantly more for women.

Factors associated with nephropathy

Normal CC range is between 80−140 mL/min. Healthy Tunisian patients (controls) had creatinine clearance 104.5 (4.7) mL/min (Table 3). All Type 2 DM patients had lower CC (78.7 (2.8) mL/min). In the same manner, when the Type 2 DM group with nephropathy was excluded, CC level of Type 2 DM patients without nephropathy was 78.6 (3) mL/min. Difference between CC in Type 2 DM with/without nephropathy, and healthy volunteers was significant ($p < 0.0001$, Table 3). The comparison of other renal parameters

Table 3: Comparison of kidney factors between Type 2 diabetes mellitus (DM) patients and healthy volunteers

	All Type 2 DM		
	Yes	**No***	***p***
Creatinine (µmol/L)	68.3 (2)	67.4 (0.7)	0.6640
Urea (mmol/L)	4.81 (0.2)	4.5 (0.1)	0.2017
Microalbuminuria (g/mol)	6.8 (1.3)	5.1 (0.4)	0.1820
Creatinine clearance (mL/min)	78.7 (2.8)	104.5 (4.7)	< 0.0001
	Type 2 DM without nephropathy		
	Yes	**No***	***p***
Creatinine (µmol/L)	65.9 (1.6)	67.4 (0.7)	0.3740
Urea (mmol/L)	4.7 (0.2)	4.5 (0.1)	0.4347
Microalbuminuria (g/mol)	7.2 (1.5)	5.1 (0.4)	0.1344
Creatinine clearance (mL/min)	78.6 (3)	104.5 (4.7)	< 0.0001

*No indicates healthy volunteers. Data are means (SE).

including urea, creatinine and microalbuminuria for Type 2 DM with/without nephropathy and healthy volunteers were not statistically significant (Table 3).

Table 4 reveals that the mean age of Type 2 DM patients with nephropathy was 62.7 (4) years. The clinical characteristics of this group and Type 2 DM without nephropathy group are given in Table 4. The serum levels of creatinine and urea in the Type 2 DM with nephropathy group were statistically significantly higher than the Type 2 DM without nephropathy group. Family history of diabetes was also higher in the Type 2 DM with nephropathy group than in the Type 2 DM without nephropathy group. Seventy-five per cent of Type 2 DM patients with nephropathy were taking antihypertensives without significant increase of macrovascular disease. Twenty-five per cent of Type 2 DM patients with nephropathy were taking lipid-lowering agents. This percentage was significantly different from the percentage of Type 2 DM patients without nephropathy. Type 2 diabetes mellitus patients with nephropathy had detectable retino-pathy but the difference was on the border of statistical significance. No significant differences in polyneuritis were noted between the two groups (Table 4).

Although 63% of diabetics taking sulfonylurea were Type 2 DM with nephropathy, 54% and 8% of Type 2 DM diabetics taking biguanides and glitazones, respectively, were Type 2 DM patients without nephropathy. Differences between groups for each hypoglycaemic therapy were statistically significant (Table 4). The other oral agents as well as insulin therapy were not different between Type 2 DM patients with/without nephropathy (Table 4). In addition, use of angiotensin converting enzyme (ACE) inhibitor therapy was not different between the two diabetic groups.

When we focus on women with diabetic nephropathy, we found that urea and creatinine levels were significantly increased compared with women without nephropathy (Table 5). Seventeen per cent of these women with diabetic nephropathy smoked and probably had underlying genetic risk factors. The majority of the women were taking anti-hypertension agents (83%), as well as lipid-lowering agents (33%). Differences between diabetic women with/without nephropathy in terms of drugs taken were statistically significant (Table 5). Diabetic women with nephropathy had detectable macrovascular disease and polyneuritis but the difference was on the border of statistical significance.

Creatinine and urea were found to be significantly correlated in Pearson analysis for all diabetics (Table 6A) as well as for women (Table 6B). These kidney markers were also associated in the two groups. Microalbuminuria and urinary CC were not correlated in all Type 2 DM patients and in women (Table 6). However, these latter parameters were associated only for the diabetic women cohort (Table 6B).

Urinary CC and diabetes duration in Type 2 DM patients with/without nephropathy are shown in Fig. 1. Diabetic pa-tients with nephropathy showed a comparable mean (SE) of CC [79.1 (8.7) mL/min] and diabetics without

Table 4: Factors associated with nephropathy

	Nephropathy		
	Yes	**No**	***p***
Mean (SE)			
Age (years)	62.7 (4)	58.9 (1.1)	0.3048
BMI (kg/m^2)	32.1 (2.5)	30.3 (0.7)	0.4062
Venous plasma glucose (mmol/L)	9.9 (1.5)	8.6 (0.4)	0.2848
HbA_{1c} (%)	8.5 (0.8)	7.4 (0.3)	0.1342
Duration of disease (Years)	10.25 (2.8)	10.6 (1)	0.8972
Systolic blood pressure (mmHg)	140 (10.5)	135.8 (3.2)	0.6596
Diastolic blood pressure (mmHg)	70 (4.9)	77.8 (2)	0.1898
Triglycerides (mmol/L)	1.7 (0.2)	1.5 (0.07)	0.2497
Total cholesterol (mmol/L)	4.9 (0.2)	4.7 (0.1)	0.2598
High density lipoprotein (HDL, mmol/L)	1.12 (0.1)	1.16 (0.05)	0.8145
Low density lipoprotein (LDL, mmol/L)	3.1 (0.2)	2.8 (0.1)	0.2993
Iron (µmol/L)	13.8 (1.2)	15.8 (0.7)	0.3596
Aspartate aminotransferase (AST, IU/L)	18.8 (1.6)	19.8 (0.9)	0.7144
Alanine aminotransferase (ALT, IU/L)	28 (2.5)	27.4 (1.3)	0.8637
Creatinine (µmol/L)	77.6 (5.7)	67.7 (0.96)	0.0049
Urea (mmol/L)	5.7 (0.6)	4.6 (0.2)	0.0480
Microalbuminuria (g/mol)	3.9 (0.4)	6.9 (1.5)	0.5193
Creatinine clearance (mL/min)	79.1 (8.7)	82.7 (4.1)	0.7563
White blood cells (10^3/mm^3)	5.9 (1)	6.9 (0.2)	0.2186
Haemoglobin (g/dL)	11.9 (0.6)	12.5 (0.1)	0.1285
Platelets (10^3/mm^3)	225.8 (24.1)	275.1 (8.6)	0.0710
Per cent patients with feature			
Macrovascular disease	13	9	
Retinopathy	38	26	0.0954
Polyneuritis	38	37	1.0000
Family history	88	75	0.0289
Cigarette smoking	13	8	0.3562
Insulin therapy	50	54	0.6711
Sulfonylurea therapy	63	45	0.0159
Glinides therapy	0	2	0.4773
Biguanides therapy	38	54	0.0333
Glitazones therapy	0	8	0.0115
ACE inhibitor therapy	13	14	1.0000
Anti-hypertensives (Not ACE inhibitor)	75	37	< 0.0001
Lipid-lowering agents	25	8	0.0023

ACE: Angiotensin-converting enzyme. Data are means (SE) or percentage.

nephropathy [82.7 (4.1) mL/min] (Table 4). The intra-groups variance is illustrated in Fig. 1A. This deficient urinary CC in diabetics is independent from disease duration (Fig. 1B).

Figure 2 shows the GFR estimated according to the MDRD formula in diabetic patients without nephropathy. Mean GFR within this group was 76 mL/min/1.73m^2. Table 7 indicates the GFR percentages in all Type 2 DM patients without nephropathy, by gender. Of Type 2 DM patients without nephropathy, 1.5% presented with a GFR < 60 mL/min/1.73 m^2 (Stage 3: mild to moderate) indicating a probable kidney malfunction. Seventy-six per cent had GFR between 60 and 89 mL/min/1.73 m^2 (Stage 2: mild). In contrast, only 22.5% had a GFR over 90 mL/min/1.73 m^2 [Stage 1: increased and optimal] (Table 7). Gender differences were also noticed.

Differences between GFR of Type 2 DM patients with/without nephropathy were not significant (Fig. 3) except for men. Intra-group GFR variance was significant for the entire cohort and for women (Fig. 3). However, intra-group GFR variance was not significant in the male group (Fig 3). Regarding diabetic complications associated with nephropathy, the prevalence of retinopathy in Type 2 DM patients without nephropathy is close to that of Type 2 DM patients with nephropathy for the entire cohort, women but not for men. In addition, the inter-group variance within diabetics without nephropathy and retinopathy was reduced (Fig. 3).

Table 5: Factors associated with nephropathy for women

	Nephropathy		
	Yes	**No**	***p***
Mean (SE)			
Age (years)	62.7 (4)	58.9 (1.1)	0.3048
BMI (kg/m^2)	32.1 (2.5)	30.3 (0.7)	0.4062
Age (years)	57.2 (2)	58.4 (1.3)	0.7807
BMI (kg/m^2)	34.03 (3)	31.2 (0.7)	0.2491
Venous plasma glucose (mmol/L)	8.4 (1.3)	8.7 (0.5)	0.8296
HbA_{1c} (%)	7.7 (0.8)	7.4 (0.3)	0.7020
Duration of disease (Years)	10.33 (3.6)	11.1 (1.2)	0.8226
Systolic blood pressure (mmHg)	132 (3.7)	134.6 (3.7)	0.8050
Diastolic blood pressure (mmHg)	74 (6)	78.5 (1.8)	0.4116
Triglycerides (mmol/L)	1.72 (0.24)	1.5 (0.08)	0.3643
Total cholesterol (mmol/L)	5 (0.3)	4.7 (0.1)	0.4704
High density lipoprotein (HDL, mmol/L)	1.14 (0.15)	1.11 (0.4)	0.8323
Low density lipoprotein (LDL, mmol/L)	3.1 (0.25)	2.8 (0.1)	0.4855
Iron (µmol/L)	14.4 (1.6)	15.4 (0.7)	0.6517
Aspartate aminotransferase (AST, IU/L)	18.6 (2.2)	20.1 (1.1)	0.6685
Alanine aminotransferase (ALT, IU/L)	28.4 (3.3)	27.4 (1.6)	0.8308
Creatinine (µmol/L)	71.8 (3.3)	65.7 (0.8)	0.0372
Urea (mmol/L)	5.9 (0.6)	4.4 (0.2)	0.0225
Microalbuminuria (g/mol)	3.8 (0.6)	7.63 (1.7)	0.5311
Creatinine clearance (mL/min)	73.4 (10)	84.4 (4.7)	0.4694
White blood cells (10^3/mm^3)	6.1 (1.3)	6.7 (0.2)	0.4460
Haemoglobin (g/dL)	11.9 (0.8)	12.4 (0.1)	0.2386
Platelets (10^3/mm^3)	236.4 (26.5)	278.9 (9.9)	0.1704
Per cent patients with feature			
Macrovascular disease	17	8	0.0872
Retinopathy	17	25	0.2243
Polyneuritis	50	38	0.1171
Family history	83	58	0.0002
Cigarette smoking	17	4	0.0056
Insulin therapy	67	51	0.0310
Sulfonylurea therapy	50	45	0.5711
Glinides therapy	0	2	0.4773
Biguanides therapy	50	57	0.3950
Glitazones therapy	0	8	0.0115
ACE inhibitor therapy	17	13	0.5525
Anti-hypertensives (Not ACE inhibitor)	83	36	< 0.0001
Lipid-lowering agents	33	6	< 0.0001

ACE: Angiotensin-converting enzyme. Data are means (SE) or percentage.

Type 2 diabetes mellitus patients without nephropathy including those with/without retinopathy have similar GFR as patients with nephropathy [no statistical differences for the entire cohort and for women] (Fig. 3). However, this is not the case for men.

Table 6: Correlation and associations between kidney factors in all diabetics (A), and in women (B)

A

		Nephropathy	
Correlation		**Yes**	**No**
Creatinine/urea	***R***	0.53	0.53
	p	NS	< 0.0001
Microalbuminuria/creatinine clearance	***R***	-0.52	-0.13
	R	-0.52	-0.13
	p	NS	NS
Regression			
Creatinine/urea	R^2	0.28	0.28
	F	2	20.15
Microalbuminuria/creatinine clearance	R^2	0.27	-0.14
	F	1.47	0.31

B

		Nephropathy	
Correlation		**Yes**	**No**
Creatinine/urea	***R***	-0.03	0.54
	p	0.96	0.0002
Microalbuminuria/creatinine clearance	***R***	-0.97	-0.08
	p	0.15	0.57
Regression			
Creatinine/Urea	R^2	< 0.0009	0.29
	F	0.001	17
Microalbuminuria/creatinine clearance	R^2	0.95	0.008
	F	17.94	0.33

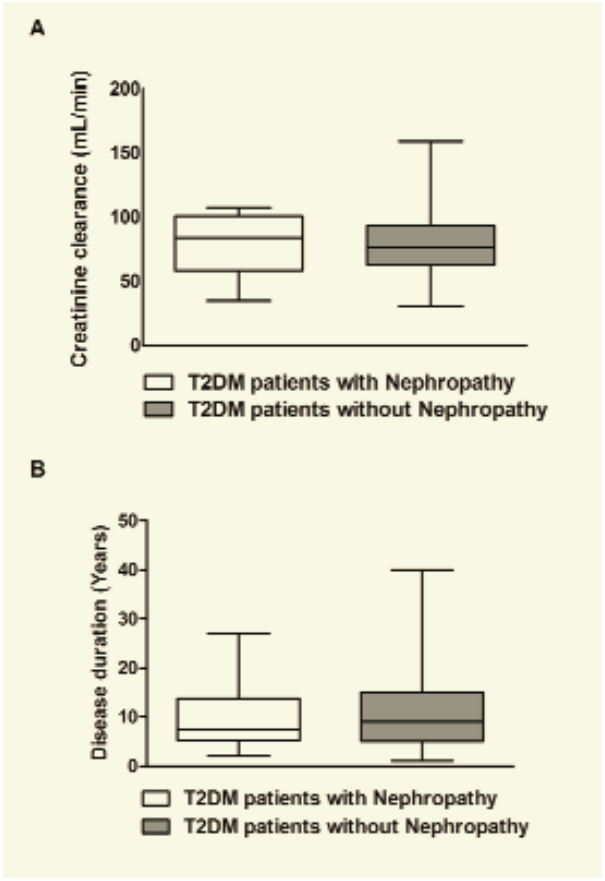

Fig. 1: The creatinine clearance level is independent of diabetes duration. (A) Means of creatinine clearance are comparable between Type 2 diabetes mellitus (DM) patients with or without nephropathy. (B) Means of diabetes duration are comparable between Type 2 DM patients with or without nephropathy.

Table 7: Repartition of glomerular filtration rate (GFR) percentage in Type 2 diabetes mellitus (DM) patients without nephropathy

(mL/min/1.73 m^2)	**Men**	**Women**	**Type 2 DM without nephropathy (%)**
GFR < 60	0	2	1.5
60 ≤ GFR ≤ 69	0	10	8
70 ≤ GFR ≤ 79	9	23.5	21
80 ≤ GFR ≤ 89	18	53	47
90 ≤ GFR ≤ 100	73	11.5	22.5

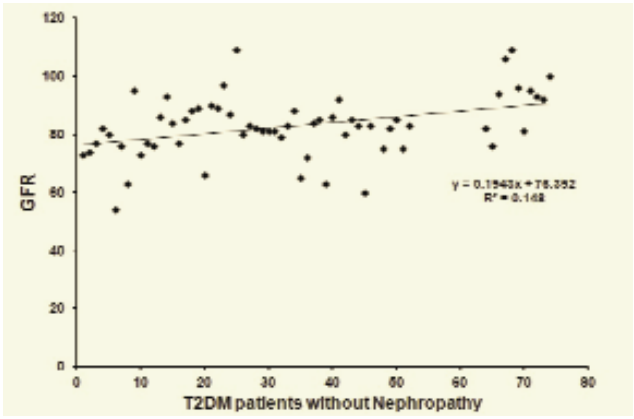

Fig. 2: The glomerular filtration rate (GFR) heterogeneity in Type 2 diabetes mellitus (DM) patients without nephropathy.

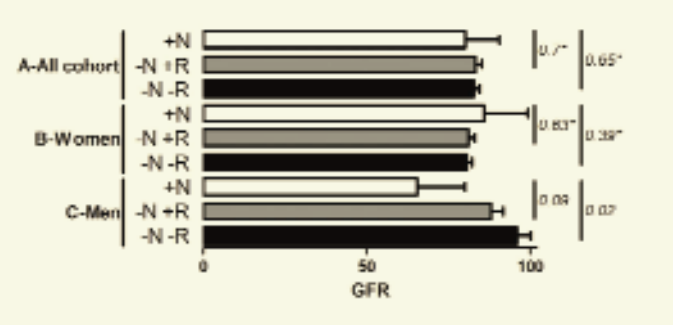

Fig. 3: The glomerular filtration rate (GFR) **i**n all Type 2 diabetes mellitus (DM) patients (A), in Type 2 DM women (B) and in Type 2 DM men (C).

+N: with nephropathy, -N: without nephropathy, +R: with retinopathy, -R: without retinopathy. *p*-values are showed in italic. * indicates significant differences in variances.

DISCUSSION

Emerging studies demonstrate that Type 2 DM patients have chronic kidney disease more than twice that of Type 1 diabetic patients (17). Moreover, 30 to 50% of long-standing Type 2 DM patients will develop nephropathy (18). In this condition, monitoring Type 2 DM patients for nephropathy becomes vital to avoid ESRD.

In these Type 2 DM Tunisian patients from Sousse, the BMI was 30.5 (0.7) kg/m^2 with an HbA_{1c} of 7.5 (0.2)%. Body mass indices were consistent with the study of Bouzid *et al* performed with Type 2 DM Tunisian patients from Tunis. Indeed, the population from Tunis had a BMI of 28.8 (5.5) kg/m^2 with an HbA_{1c} of 10.8 (2.3) % (19). Contrary to our patients who were frequently obese, Bouzid's patients were not obese (19).

Because different studies have reported the increased potential of Type 2 DM Tunisian patients to renal failure, 19.8% (19) and 36.9% (20) of Type 2 DM Tunisian patients have renal failure, we monitored GFR in the Type 2 DM patients. Seventy-six per cent of Type 2 DM patients without nephropathy belonged to stage 2 chronic kindney disease. This stage is characterized by normal ranges of blood pressure as well as albumin excretion (21). However, in this stage, kidney function starts to decrease (22). This result should be confirmed by urinalysis, imaging or biopsies (6). However, the GFR calculation alone could be sufficient for Type 2 DM patients with nephropathy (1.5%; GFR stage 3). For this stage, GFR may be supranormal but declining (21). This means that preventive treatment should be started. These patients should be monitored regularly for their GFR and should contact a nephrologist to slow progression of kidney disease. Moreover, GFR measures should be performed especially because normal albuminuria may denote incipient GFR reduction in Type 2 DM patients (23). Support should be provided for patients at GFR stages 2 and 3 especially if they are overweight or obese. This support becomes important for the Tunisian obese population that frequently demonstrated the metabolic syndrome (24). The National Kidney Foundation (NKF) recommends decreasing BMI to 18.5−24.9 kg/m^2 for patients with diabetes (25). Moreover, a protein restriction to 0.8 g/kg seems reasonable for these patients (21). In addition, diabetic drugs should be correctly selected (6, 26). For example, sulfonylureas should not be associated with other medications to lessen the plasma levels of free sulfonylureas (27−28).

Type 2 diabetes mellitus Tunisian patients with nephropathy should be monitored closely. Indeed, HbA_{1c} should decrease from the noted value (8.5%) and be maintained < 7% (2, 6). Continuous monitoring of blood pressure (29−30), serum lipids and albumin rates should be checked to avoid more complications of Type 2 DM (7, 31).

Smoking cessation may be beneficial for Type 2 DM Tunisian patients, particularly men (32−33). In addition, patients should be on a special low-protein diet to slow progression to end-stage renal failure and dietary restrictions to decrease their obesity (34–36). Moreover, physicians may subscribe more lipid-lowering agents like statins to reduce the LDL levels to < 2.59 mmol/L (37).

The susceptibility of Tunisians from the region of Sousse to diabetic nephropathy, indicated by GFR of stage 2 and 3, necessitates treatment with ACE inhibitors (38) or angiotensin receptor blockers to prevent or to delay progression (1, 39–41). These drugs could be efficient especially because the renin-angiotensin-aldosterone system genotypes and haplotypes affect the susceptibility of Tunisian Type 2 DM populations to nephropathy (42). The administration of other drugs including diuretics, beta-blockers, and calcium-channel blockers may also be beneficial for certain patients with diabetic nephropathy (1).

The screening for diabetic retinopathy should also be performed annually in Type 2 DM Tunisian patients from Sousse especially after the clinical studies demonstrating that retinopathy could indicate a potential development of nephropathy (43−45). Indeed, Type 2 DM patients with retinopathy and proteinuria frequently have nephropathy (45). Emerging studies exploring these complications in Type 2 DM support the association of retinopathy and nephropathy (45). However, the absence of retinopathy does not exclude nephropathy (45−46).

CONCLUSION

This study demonstrates the susceptibility of Tunisian Type 2 DM patients from Sousse to nephropathy. The regular monitoring of these patients should delay the onset of this co-morbidity. Moreover, the achievement of the ADA targets should be very helpful as demonstrated in other populations (7, 47). Finally, frequent GFR measurements should be performed.

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