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NON-DIABETIC RENAL DISEASE IN CROATIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Aims: Our study aimed to examine the prevalence of non-diabetic renal disease in selected patients with type 2 diabetes mellitus and to determine important risk factors for non-diabetic renal disease.

Methods: We conducted retrospective analysis of clinical, laboratory and pathohistological data of type 2 diabetes mellitus patients in whom renal biopsies were performed from January 2004 to February 2013 at Dubrava University Hospital Zagreb Croatia (n=80).

Results: According to renal biopsy findings, isolated diabetic nephropathy was found in 46.25%, non-diabetic renal disease superimposed on diabetic nephropathy in 17,5% and isolated non-diabetic renal disease in 36,25% of the patients. The most common non-diabetic renal diseases found were: membranous nephropathy, followed by IgA nephropathy and focal segmental glomerulosclerosis. In univariate analysis shorter duration of diabetes, independence of insulin therapy, lower levels of HbA1c and absence of diabetic retinopathy were found to be significant clinical predictors of non-diabetic renal disease. In multivariate analysis only independence of insulin therapy (OR 4.418, 95%CI=1.477-13.216) and absence of diabetic retinopathy (OR 5.579, 95%CI=1.788-17.404) were independent predictors of non-diabetic renal disease.

Conclusions: This study confirmed usefulness of renal biopsy in patients with type 2 diabetes mellitus, due to the high prevalence of non-diabetic renal disease found. Since non-diabetic renal disease are potentially curable, we should consider renal biopsy in selected type 2 diabetes mellitus patients with renal involvement, especially in those with absence of diabetic retinopathy and independence of insulin therapy.

KEYWORDS: type 2 diabetes mellitus, diabetic nephropathy, diabetic retinopathy, renal biopsy, non-diabetic renal disease

INTRODUCTION

The incidence and prevalence of type 2 diabetes mellitus (T2DM) are increasing and becoming one of the major health care problems in the world [1, 2]. Diabetic nephropathy (DN) is one of the major complications of diabetes mellitus and is reported as the leading cause of the end-stage renal disease (ESRD) worldwide [1, 3]. The diagnosis of DN is mostly clinical, based on duration of T2DM and the presence of retinopathy, neuropathy and other chronic complications, proteinuria and slowly progressing azothemia. This kind of diagnostic approach has been constantly challenged, due to the fact that other non-diabetic renal diseases (NDRD) have been found in T2DM patients. The prevalence of other biopsy-proven glomerular, tubulointerstitial and /or vascular diseases in T2DM in reported studies [4-27] varies considerably, ranging from 8% [4] to 93.5% [5]. This depends on the selection criteria, indications and availability of renal biopsy as well as on the population investigated. Despite the fact that NDRD in selected T2DM patients is not uncommon and renal biopsy is the only tool to absolutely identify DN or NDRD, the role of renal biopsy in T2DM patients with signs and symptoms of renal disease remains controversial. The findings of NDRD could have major therapeutic and prognostic implications, since the majority of glomerular and tubulointerstitial diseases are treatable, even remittable, which is quite different from DN. This is supported by the results of a recent study, which showed that the patients with NDRD have significantly better renal outcomes compared to patients with DN only [7]. The results of previous studies on discriminatory factors between DN and NDRD are not uniform, and there are differences in study populations and selection criteria [4-27]. The purpose of this study was to evaluate the indications of renal biopsy and to determine predictors of NDRD and DN in Croatian patients with T2DM referred to our center. In our center the majority of adult native renal biopsies in Croatia are performed, and our results were recently published [28].

SUBJECTS, MATERIALS AND METHODS

Patients and methods

The present study was conducted by reviewing the medical records of T2DM patients who underwent percutaneous renal biopsy in Dubrava University Hospital, Zagreb, Croatia from January 2004 to February 2013. All patients were diagnosed at the time of biopsy with T2DM as defined by the WHO, ADA and EDA [1, 29, 30]. Biopsy indications were uniform throughout the study period and were based on clinically strong suspicion of NDRD and included one or more of the following factors: heavy proteinuria or nephrotic syndrome, renal failure (acute, rapidly progressive or unexplained chronic), absence of diabetic retinopathy, findings of persistent glomerular hematuria, clinical or laboratory findings of systemic autoimmune disease or hematologic malignancy. The following clinical data were collected for each patient: age at the time of the biopsy, gender, duration of diabetes prior to biopsy, presence of hypertension (including systolic, diastolic and mean arterial pressure), presence of diabetic retinopathy, presence of glomerular hematuria, history of insulin therapy. Laboratory data collected at the time of the biopsy were as follows: urinalysis, serum creatinine, serum albumin and proteins, hemoglobin A1c (HbA1c), maximal 24-hour proteinuria, estimated glomerular filtration rate (EGFR, determined by the CKD-EPI formula). Ultrasound was used to determine kidney size and enlarged kidneys were defined as >12cm on the longitudinal axis bilaterally. Hypertension was defined as systolic blood pressure≥140mmHg or diastolic blood pressure≥90mmHg or antihypertensive medications being taken by the patient. Diabetic retinopathy was diagnosed by direct ophthalmoscopy performed by an ophthalmologist. Hematuria was defined as >3 red blood cells per high power microscope field in a centrifuged urine sample. Percutaneous renal biopsy using kidney biopsy gun (16G) was performed after obtaining a signed informed consent from each patient. Renal tissue obtained was sent for light, immunofluorescence and electron microscopic examination routinely. All biopsies were reviewed by two experienced and independent pathologists.

Only biopsies suitable for definitive diagnosis were included in the study. DN was diagnosed based on the presence of mesangial expansion and diffuse intercapillary glomerulosclerosis with or without Kimmelstiel-Wilson nodules, basement membrane thickening and exudative lesions, such as fibrin caps, capsular drop or hyaline thrombi [31]. Based on the biopsy findings, patients were divided into three basic groups: patients with isolated DN, patients with NDRD superimposed on DN (mixed lesions) and patients with isolated NDRD. Because we planned to investigate predictors for DN and for NDRD, we furthermore created two more classification groups, which distinguished patients on the basis of having DN (DN vs. non DN patients) and on the basis of having NDRD (NDRD vs. non NDRD patients).

Statistical analysis

Statistical analyses were performed using PASW Statistics (version 18.0, SPSS Inc. Chicago, IL, USA). Normally distributed data were expressed as mean±SD, skewed data as median with interquartile range and categorical data as frequency (%). Differences between groups were evaluated by Student t-test or ANOVA for normally distributed data, by Mann-Whitney U test or Kruskal-Wallis test for skewed data and by chi-square (χ^2)-test for categorical data. Multiple logistic regression using forward stepwise method was performed to determine independent predictors for DN and for NDRD, including all covariates with a p-value of <0.05 in univariate analysis. Receiver operating characteristics (ROC) curves were constructed for significant variables of NDRD and DN by plotting sensitivity vs. 1-specificity and the areas under the ROC curves (AUC) were calculated for determining sensitivity and specificity of predictors. Significance was evaluated using a two-sided p value of <0.05.

RESULTS

80 patients with T2DM were included in this study. Mean age at biopsy was 59.5±9.8 years, 70% of patients were male and median duration of diabetes was 10 years (ranging from

0-i.e. newly diagnosed disease to 39 years). The baseline clinical and laboratory data collected are shown in Table 1.

The most common indication for renal biopsy was nephrotic syndrome (80%) and in 75% of patients there was renal failure (acute, rapidly progressive or unexplained chronic). In 43 patients NDRD was found on renal biopsy (29 patients had isolated NDRD and 14 patients had mixed lesions of NDRD and DN). In 51 patients DN was found (37 had isolated DN and 14 patients had mixed lesions). NDRD found in our patients are shown in Table 2. The most common NDRD was membranous nephropathy in 9 patients (20.9%), followed by IgA nephropathy in 8 patients and focal segmental glomerulosclerosis (FSGS) in 8 patients also.

Univariate analysis of our basic classification groups (DN vs. mixed lesions vs. NDRD, Table 1.) demonstrated only significant difference regarding insulin therapy ($p<0.000$) and presence of diabetic retinopathy ($p<0.000$).

Classification groups II (DN vs. non DN) comparison showed that patients having DN had significantly longer duration of diabetes ($p<0.000$), were more common on insulin therapy ($p<0.000$), had higher serum creatinine ($p=0.012$), lower EGFR ($p=0.014$) and had more common diabetic retinopathy ($p<0.000$) as compared with patients not having DN (Table 3.).

Classification groups III (NDRD vs. non NDRD) univariate analysis revealed that patients with NDRD had shorter duration of diabetes ($p=0.001$), were less common dependent on insulin therapy ($p<0.000$), had lower serum HbA1c ($p=0.006$) and had less frequently diabetic retinopathy ($p<0.000$) as compared with patients not having NDRD (Table 3.). Multivariate logistic regression analysis was used to determine risk factors associated with DN and NDRD, and variables found statistically significant in univariate analysis were used. The results are summarized in Table 4. Significant risk factors for DN were duration of diabetes prior to biopsy (OR 1.183; 95%CI=1.070-1.308; $p=0.001$) and presence of diabetic

retinopathy (OR 24.531; 95%CI=2.862-210.278; p=0.004). Significant risk factors for NDRD were independence of insulin therapy (OR 4.418, 95%CI=1.477-13.216) and absence of diabetic retinopathy (OR 5.579, 95%CI=1.788-17.404). We evaluated sensitivity and specificity of those factors in prediction of DN and NDRD in ROC analysis, and results are summarized in Table 5. For DN duration of diabetes of more than 7 years (cut-off value determined by ROC analysis) showed highest AUC. When including duration of diabetes variable as categorical with cut-off value of 7 years, in the logistic regression model, OR for this variable in the prediction of DN was found to be 13.074 (95%CI=3.459-48.859). The AUC curves of the predictors for NDRD are shown in Figure 1.

DISSCUSION

Diabetic nephropathy is the most common cause of ESRD worldwide [1, 3], while diagnosis of DN is frequently based on clinical criteria exclusively and patients with potential NDRD are often overlooked. Comparison of clinical diagnostic criteria and histologic findings of DN is usually not directly tested in recent studies [4-27], as well as in our study. This is probably due to the fact that the research priority is finding of predisposing factors of NDRD and not of DN. Only Biesenbach et al. found high sensitivity of clinical diagnosis in the prediction of DN [32]. The limitation of this study is that it is mostly *post mortem* study, and the advantage is that there aren't any usual biases in selection criteria.

In most cases NDRD are treatable and even curable diseases and therefore it is of great importance to diagnose and differentiate NDRD among T2DM patients with renal signs and symptoms. The prevalence of NDRD in published studies varies widely ranging from 8% to 93.5% [4-27]. In our study it was 53.8%, which is most similar to findings of Mou S et al. [8]. Due to the fact that it is not ethical to perform kidney biopsy in all T2DM patients with renal involvement, we will never know the true prevalence of NDRD as well as that of DN in T2DM patients. In 1992, Waldherr et al. performed autopsy in 205 T2DM patients and found

NDRD in 0.4% and DN in 79% of the cases [33]. This post mortem study, as well as that of Biesenbach et al. [32], which included mostly ESRD patients, probably led to underestimation of NDRD in T2DM. The reason for this is that in ESRD, some cases of NDRD can't be distinguished from DN, there is only advanced glomerular and tubulointerstitial scarring. This conclusion is supported by the findings of Biesenbach et al., which didn't find any NDRD, but only DN and vascular nephropathy in their study [32].

The large variation in the reported prevalence of NDRD is mostly the results of the different criteria for renal biopsy and possibly due to geographical and ethnic differences also. In large majority of reported studies, as well as in ours, the main indication for renal biopsy was clinically thorough suspicion of NDRD. This usually includes any renal function abnormality and/or urine sediment abnormality (proteinuria, glomerular hematuria), which is not consistent with the typical course of T2DM [29, 31]. Majority of studies also report absence of diabetic retinopathy as the biopsy criteria [5-7, 9, 11-16]. Although relatively uniformly defined, there were minor differences among studies, regarding indications for biopsy, and also in some studies threshold criteria were not clearly defined. Common indications for renal biopsy in reported studies included acute or rapidly progressive renal failure [5, 6, 7, 9, 11-13, 16], proteinuria [5-11, 13-17], glomerular hematuria [5-7, 9, 11-13, 16] and absence of diabetic retinopathy [5-7, 9, 11-13, 15, 16] as well as shorter duration of T2DM [5, 6, 8, 13]. This is in consistence with our indications for renal biopsy. Differences in selection criteria imply the necessity for standardization of renal biopsy criteria in T2DM. This refers in particular on renal function parameters (creatinine, EGFR) and proteinuria. In reported studies there is usually no clear threshold level of creatinine or EGFR bellow which biopsy is not performed, only in study of Zhou J et al. biopsy was contraindicated if serum creatinine was above 442μmol/l [10]. The serum creatinine level and/or EGFR should not be of importance when there is acute or rapidly progressive renal failure. Regarding proteinuria there are

usually not clearly defined threshold levels for renal biopsy, only a few researchers performed biopsy only when proteinuria was above 1g/24 hours [15, 17, 19]. Age, duration of diabetes and renal size on ultrasound are also factors that need to be uniformly defined as biopsy criteria. Some authors excluded patients older than 65-70 years [8, 15], and also if the duration of T2DM was longer than 10 years [8]. We believe that biopsy is probably of no importance when there is advanced chronic renal insufficiency with smaller, shrunken kidneys on ultrasound (at which EGFR level is still to be determined by future studies), because of expected findings of diffuse global glomerulosclerosis and tubular atrophia with interstitial fibrosis, without any reference to underlying disease.

The incidence of NDRD in T2DM is mostly dependent on the threshold and criteria for performing renal biopsy and unifying will enable to extrapolate the findings of smaller local studies to larger populations, as well as to compare different populations and to perform meta-analyses. Low threshold for biopsy probably explains high prevalence of NDRD in certain reports [34] and leads to overestimation of the NDRD prevalence in T2DM patients, and the opposite is the case in high thresholds for biopsy.

The most common NDRD reported are glomerulonephritides [7, 8, 10, 13, 14, 17, 21, 22] which is consistent with our results. In some reports the most common NDRD was interstitial nephritis [6, 11]. Among glomerulonephritides, the most common found was IgA nephropathy [10, 13, 14, 17, 22], and in some reports it was FSGS [8, 21] or membranous nephropathy [7]. We found membranous nephropathy as the most common glomerulonephritis and as the most common NDRD also, followed by IgA nephropathy and FSGS. The complete and correct diagnosis of glomerular diseases depends on the use of immunofluorescence (IF) and electron microscopy (EM) in the analysis of renal biopsy. Immunofluorescence microscopy is crucial in the diagnosis of IgA nephropathy and also in differentiation between types of crescentic glomerulonephritides. EM is necessary for the

diagnosis of minimal change disease and also for the differentiation between primary and secondary FSGS. EM in some cases can also help diagnosing early DN (on the bases of thickened glomerular basement membranes). Some of the reported studies did not routinely use EM [6, 9, 11] and in our study EM was routinely used. We think this is one of the advantages of our study. In two studies vascular nephropathy is separated as a distinct entity, independently of DN and NDRD [32, 35]. In most studies, as well as in our study, vascular nephropathy was a part of NDRD spectrum (hypertensive nephrosclerosis). The problem with this diagnosis is that some authors believe that some pathohistological changes are concomitantly part of DN and also of hypertensive nephrosclerosis.

Due to the fact that there is still no general agreement on selection criteria for renal biopsy in T2DM patients, it is important to be able to identify clinical predictors of NDRD. Wide variation also exists in reported significant risk factors for NDRD [6, 7, 8, 10, 13, 14, 17, 21, 22]. In our analysis of important predictors, we divided our patients into three classification groups. The reason for this is the fact that there is not always a clear distinction between DN and NDRD, i.e. that there are patients with mixed lesions (NDRD superimposed on DN) which is consistent with most reported studies [5-7, 11-13, 16, 17, 19, 20, 21, 23, 27]. Some studies didn't find patients with mixed lesions [8, 10, 15, 24], while others reported no isolated NDRD [9, 14, 22]. It is difficult to determine the cause of lack of isolated NDRD or mixed lesions respectively in some studies. The potential causes include still uniform pathohistologic criteria for DN, lack of IF or EM use in some studies and also earlier mentioned different renal biopsy thresholds. We think that basic classification group I (isolated DN, mixed lesions, isolated NDRD; Table 1.) should serve only in descriptive purposes and not for the analysis of potential risk factors. The analysis in classification group III (NDRD vs. non-NDRD) is the most important for determination of risk factors because the presence of NDRD is a potential specific treatment target, whether there is isolated NDRD or

NDRD superimposed on DN (mixed lesions). We found in univariate analysis that shorter duration of T2DM, independence of insulin therapy, lower HbA1c and absence of diabetic retinopathy were significant risk factors for NDRD. In multivariate analysis independence of insulin therapy and absence of diabetic retinopathy were found significant independent predictors for NDRD. Absence of diabetic retinopathy is reported as significant predictor for NDRD in majority of studies in univariate analysis [7, 8, 10, 13, 21, 22], and in some of them also in multivariate analysis [7, 8, 10]. Minority of studies didn't find absence of diabetic retinopathy as significant predictor for NDRD [6, 14]. This study therefore confirms the accepted view that the absence of diabetic retinopathy in T2DM patients with renal involvement should raise the possibility of NDRD, hence the renal biopsy. In our study, sensitivity and specificity of absence of diabetic retinopathy in prediction of NDRD was found 73.47% and 77.42% respectively. In comparison, Mou S et al. found sensitivity and specificity of 72.7% and 91.7% [8], and Wong TY et al. of 81.8% and 70.8% respectively [13]. Independence of insulin therapy was investigated in a few studies and was found as a significant predictor of NDRD only in the study of Wong TY et al. [13]. For the insulin therapy independence in prediction of NDRD, we didn't find reported sensitivity or specificity in published studies for comparison.

In our study, other investigated clinical and laboratory variables were not found statistically significant predictors of NDRD in multivariate analysis. In published studies, shorter duration of diabetes was found significant predictor of NDRD in multivariate analysis in a few studies [7, 10], while in others, like in ours, it wasn't found significant [6, 8, 13, 14, 17, 21, 22]. We found longer duration of diabetes as a significant predictor of DN in multivariate analysis (for duration of >7 years, OR was 13,074) and for NDRD only in univariate analysis, which is similar to Chong YB et al. [6]. For clinicians, this is probably not decisive, because the presence of DN does not exclude NDRD.

Age and gender were not significant predictors of NDRD in our study, which is consistent with the majority of findings [6, 8, 10, 13, 14, 17, 21, 22]. Only Chang TI et al. found increased age as a significant risk factor for NDRD, but only in univariate analysis [7].

Hypertension is often concomitant finding in patients with T2DM, but its predictive value of NDRD found, varies considerably. Hypertension was found as a significant predictor of NDRD in studies of Zhou J et al. [10] and Wong TY et al. [13], while other studies didn't confirm this [6-8, 14, 17, 21, 22].

Baseline morphometric variables (weight, height, body mass index) are not reported to be significant in prediction of NDRD in published studies [6-8, 10, 13, 14, 17, 21, 22], as well as in our study.

Variation exists in analysis of proteinuria as a predictor for NDRD. This is mostly due to different inclusion criteria for renal biopsy regarding proteinuria in T2DM patients. Majority of studies didn't found proteinuria significant in prediction of NDRD [6-8, 10, 13, 14, 21], similar to our results. Mak SK et al. found lower proteinuria as a significant predictor of NDRD [17], which is opposite to findings of Bi H et al., who found higher proteinuria as significant predictor of NDRD [22].

Renal excretion function (measured by serum creatinine levels and/or estimated glomerular filtration rate in reported studies) was not found significant predictor of NDRD in our study as well as in majority of studies [8, 10, 14, 17, 21, 22]. In a few studies significantly linking renal function with NDRD, findings, like with proteinuria, were opposite. Chang TI et al. [7] and Mou S et al. [8] found lower serum creatinine levels as significant predictor of NDRD, while Chong YB et al. [6] found this for higher serum creatinine. As well as in the case of proteinuria, the opposite reported significance of serum creatinine in prediction of NDRD is probably reflected by the indications for renal biopsy.

Hematuria is variably associated with NDRD. Some authors found that hematuria is infrequent in DN, while majority of glomerular diseases present with hematuria, and therefore hematuria becomes important differential indicator of NDRD vs. non-NDRD. This is supported by the results of some studies [6, 10, 17, 22]. Our results, don't support these findings, and some other authors didn't find hematuria significant as well [7, 8, 13, 14, 21].

Serum protein and albumin levels were not found significant predictors of NDRD in majority of studies [6-8, 10, 17, 21, 22], and in our study as well. Only Suzuki D et al. found lower serum protein levels as a significant predictor of NDRD [14].

We found lower serum HbA1c levels significantly associated with NDRD, but only in univariate analysis. Similar to this finding, most studies didn't find its significance in prediction of NDRD, only Zhou J et al. found it significant in multivariate analysis [10].

Other reported significant predictors of NDRD in T2DM patients include higher serum hemoglobin levels [7], higher cardiac ejection fraction, lower intima-media thickness and smaller carotid artery plaques [8].

Our study has several obvious limitations. It is a retrospective study and therefore ascertainment error, recall, informative censoring and lead-time biases cannot be avoided. Since renal biopsy in patients included in our study was performed with a strong suspicion of NDRD, biases in selecting patients is another limitation of our study. We think that the advantages of our study are routine use of immunofluorescence and electron microscopy in renal biopsy analysis, no limitations in inclusion criteria regarding age and serum creatinine and also multivariate analysis.

In conclusion, high prevalence of NDRD in our study supports the decision for biopsy, and findings of NDRD implicated specific therapeutic approach. We think that findings of our study, in conjunction with other studies, imply that signs of renal disease in all T2DM patients cannot be confidently presumed to be due to DN, and that careful individual approach to each

patient regarding renal biopsy, is required. Renal biopsy should always be considered in selected group of T2DM patients with renal involvement. Since considerable variability in investigated and reported predictors for NDRD exists, further studies are needed to determine certain, clear, unbiased renal biopsy criteria. Until then, absence of diabetic retinopathy, nephrotic range proteinuria, acute or rapidly progressive renal failure, glomerular hematuria, independence of insulin therapy and shorter duration of diabetes are proposed as risk factors of NDRD in T2DM.

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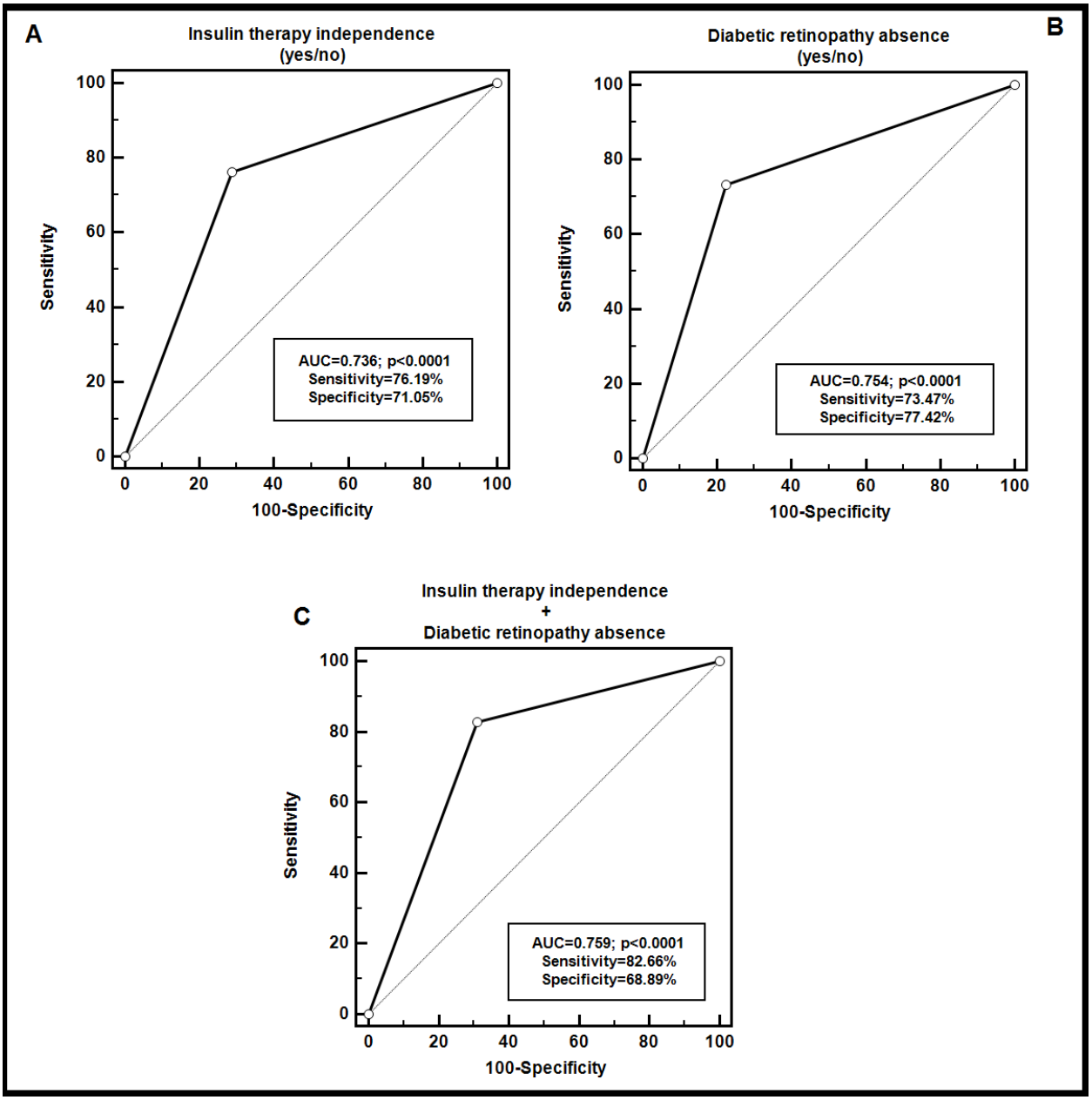
FIGURES

Figure 1. Receiver operating characteristic curves in the analysis of predictors of non-diabetic renal disease. A for independence of insulin therapy, B for absence of diabetic retinopathy and C for combination of these two variables.



TABLES

Table 1. Clinical and biochemical characteristics of the study patients in total and in classification groups I (isolated DN vs. mixed lesions vs. isolated NDRD)

	ALL	CLASSIFICATION GROUPS I		
	(n=80)	DN (n=37)	MIX (n=14)	NDRD (n=29)
Age (years)	59.5±9.8	58.9±8.8	61.9±11.4	59.1±10.4
Gender (Male)	56 (70%)	24 (64.9%)	12 (85.7%)	20 (69%)
Diabetes duration (years)	10 (1.65-16.0)	15 (9-19)	14.5 (8-20)	1.8 (0.5-6.0)
Insulin therapy	38 (47.5%)	27 (73%)	7 (50%)	4 (13.8%)*
Weight (kg)	89.5 (78.0-100.0)	90 (79-101)	90.25 (75-106)	89 (78-99)
Height (cm)	170.7±8.5	171.8±8.7	170.1±9.5	169.6±8.0
Body mass index (kg/m^2)	30.53 (27.52-33.44)	29.7 (26.12-33.63)	32.87 (28.41-36.68)	30.47 (28.13-32.77)
Hypertension	59 (73.8%)	27 (73%)	12 (85.7%)	20 (69%)
Systolic blood pressure (mmHg)	150 (130-162.5)	160 (130-170)	155 (150-170)	140 (130-160)
Diastolic blood pressure (mmHg)	90 (80-97.5)	90 (80-95)	92.5 (70-100)	80 (80-90)
Mean arterial pressure (mmHg)	106.7 (96.7-120)	113.3 (98.3-120)	116.7 (103.3-120)	103.3 (93.3-113.3)
Serum creatinine (μmol/l)	154 (119.5-227.5)	160 (136-230)	211 (139-276)	134 (105-176)
Estimated GFR (ml/minute)	37.95 (25.68-50.5)	37.99 (24.75-43.9)	27.07 (19.03-45.03)	46.83 (32.19-63.67)
24-hour proteinuria (g)	5.64 (3.35-9.75)	4.84 (3.5-8.59)	6.65 (4-14)	4.5 (2.75-11.6)
Serum albumins (g/l)	33 (28-37.5)	33 (31-38)	34.5 (21-37)	33 (26.9-37)
Hemoglobin A1c (%)	6.95 (6.4-7.9)	7.5 (6.6-8.4)	6.85 (5.2-7.2)	6.8 (6.3-7.3)
Hemoglobin A1c (mmol/mol)	52 (46-63)	58 (49-68)	51 (33-55)	51 (45-56)
ULS kidney enlargement	21 (26.6%)	12 (32.4%)	5 (35.7%)	4 (14.3%)
Nephrotic syndrome	64 (80%)	29 (78.4%)	12 (85.7%)	23 (79.3%)
Renal failure	60 (75%)	30 (81.1%)	12 (85.7%)	18 (62.1%)
Hematuria	45 (56.3%)	23 (62.2%)	6 (42.9%)	16 (55.2%)
Diabetic retinopathy	31 (38.8%)	24 (64.9%)	6 (42.9%)	1 (3.4%)*

* $p < 0.05$ (χ^2-test); GFR=glomerular filtration rate; ULS=ultrasound; DN=diabetic nephropathy; NDRD=non-diabetic renal disease; MIX=NDRD superimposed on DN.

Table 2. Non-diabetic renal disease found in our study patients

Non-diabetic biopsy-proven renal disease	All (n=43)	NDRD + DN (n=14)	NDRD only (n=29)
AL amyloidosis	1 (2.3%)	0	1 (3.4%)
Pauci-immune glomerulonephritis	2 (4.7%)	0	2 (6.9%)
Fibrilary glomerulonephritis	1 (2.3%)	0	1 (3.4%)
Focal segmental glomerulosclerosis	8 (18.6%)	3 (21.4%)	5 (17.2%)
IgA nephropathy	8 (18.6%)	3 (21.4%)	5 (17.2%)
Lupus nephritis	1 (2.3%)	0	1 (3.4%)
Minimal change disease	3 (7.0%)	1 (7.1%)	2 (6.9%)
Membranous nephropathy	9 (20.9%)	2 (14.3%)	7 (24.1%)
Membranoproliferative glomerulonephritis	1 (2.3%)	0	1 (3.4%)
Myeloma kidney	1 (2.3%)	0	1 (3.4%)
Hypertensive nephrosclerosis	5 (11.6%)	4 (28.6%)	1 (3.4%)
Postinfectious glomerulonephritis	1 (2.3%)	0	1 (3.4%)
Tubulointerstitial nephritis	2 (4.6%)	1 (7.1%)	1 (3.4%)

DN=diabetic nephropathy; NDRD=non-diabetic renal disease.

Table 3. Clinical and biochemical characteristics of the study patients in the classification groups II (DN vs. non-DN) and in the classification groups III (NDRD vs. non-NDRD)

	CLASSIFICATION GROUPS II		CLASSIFICATION GROUPS III	
	DN (n=51)	no DN (n=29)	NDRD (n=43)	no NDRD (n=37)
Age (years)	59.8±9.6	59.1±10.4	60±10.7	58.9±8.8
Gender (Male)	36 (70.6%)	20 (69%)	32 (74.4%)	24 (64.9%)
Diabetes duration (years)	15 (8-19)	1.8 (0.5-6.0)*	3 (0.5-12)	15 (9-19)*
Insulin therapy	34 (66.7%)	4 (13.8%)*	11 (25.6%)	27 (73%)*
Weight (kg)	90 (78-102)	89 (78-99)	89 (78-99.5)	90 (79-101)
Height (cm)	171.4±8.8	169.6±8.0	169.8±8.4	171.8±8.7
Body mass index (kg/m^2)	30.59 (26.12-34.2)	30.47 (28.13-32.77)	30.74 (28.13-33.25)	29.7 (26.12-33.63)
Hypertension	39 (76.5%)	20 (69%)	32 (74.4%)	27 (73%)
Systolic blood pressure (mmHg)	160 (130-170)	140 (130-160)	150 (130-160)	160 (130-170)
Diastolic blood pressure (mmHg)	90 (80-100)	80 (80-90)	90 (75-100)	90 (80-95)
Mean arterial pressure (mmHg)	113.3 (98.3-120)	103.3 (93.3-113.3)	106.7 (93.3-116.7)	113.3 (98.3-120)
Serum creatinine (µmol/l)	170 (136-249)	134 (105-176)*	141 (109-225)	160 (136-230)
Estimated GFR (ml/minute)	33.57 (21.1-44.35)	46.83 (32.19-63.67)*	37.91 (26.32-56.85)	37.99 (24.75-43.9)
24-hour proteinuria (g)	5.8 (3.6-8.7)	4.5 (2.75-11.6)	5.67 (2.76-12)	4.84 (3.5-8.59)
Serum albumins (g/l)	34 (29-38)	33 (26.9-37)	33 (26-37)	33 (31-38)
Hemoglobin A1c (%)	7.1 (6.5-8.4)	6.8 (6.3-7.3)	6.8 (6.1-7.3)	7.5 (6.6-8.4)*
Hemoglobin A1c (mmol/mol)	54 (48-68)	51 (45-56)	51 (43-56)	58 (49-68)*
ULS kidney enlargement	17 (33.3%)	4 (14.3%)	9 (21.4%)	12 (32.4%)
Nephrotic syndrome	41 (80.4%)	23 (79.3%)	35 (81.4%)	29 (78.4%)
Renal failure	42 (82.4%)	18 (62.1%)	30 (69.8%)	30 (81.1%)
Hematuria	29 (56.9%	16 (55.2%)	22 (51.2%)	23 (62.2%)
Diabetic retinopathy	30 (58.8%)	1 (3.4%)*	7 (16.3%)	24 (64.9%)*

* $p<0.05$ (Mann-Whitney test for continuous and χ^2-test for categorical variables); GFR=glomerular filtration rate; ULS=ultrasound; DN=diabetic nephropathy; NDRD=non-diabetic renal disease.

Table 4. Multivariate logistic regression analysis of diabetic nephropathy and of non-diabetic renal disease

Indicator	β-estimate	Standard error	p-value	Odds ratio	95% confidence interval
For diabetic nephropathy					
Duration of diabetes (years)	0.168	0.051	0.001	1.183	1.070-1.308
Diabetic retinopathy (yes vs. no)	3.200	1.096	0.004	24.531	2.862-210.278
For non-diabetic renal disease					
Insulin therapy (no vs. yes)	1.486	0.559	0.008	4.418	1.477-13.216
Diabetic retinopathy (no vs. yes)	1.719	0.580	0.003	5.579	1.788-17.404

Table 5. Sensitivity, specificity, positive and negative predictive values of significant variables in the prediction of diabetic nephropathy and of non-diabetic renal disease

Variable	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
Prediction of diabetic nephropathy						
Duration of diabetes (>7 years)	0.828	78.43	82.76	88.9	68.6	<0.0001
Diabetic retinopathy (yes vs. no)	0.777	58.82	96.55	96.8	57.1	<0.0001
Prediction of non-diabetic renal disease						
Insulin therapy (no vs. yes)	0.736	76.19	71.05	74.4	73.0	<0.0001
Diabetic retinopathy (no vs. yes)	0.754	73.47	77.42	83.7	64.9	<0.0001
Insulin therapy+ diabetic retinopathy (no vs. yes)	0.759	82.66	68.89	67.4	83.8	<0.0001

AUC= area under curve; PPV=positive predictive value; NPV=negative predictive value.