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Follow Up of Value of the Intrarenal Resistivity Indices and Different Renal Biomarkers for Early Identification of Diabetic Nephropathy in Type 1 Diabetic Patients

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Abstract

AIM: To evaluate intrarenal resistivity index (RI) and different biomarkers of diabetic nephropathy (DN) with clinical signs of DN and its progression over time as early detection of DN.

PATIENTS AND METHODS: This longitudinal study included 48 type 1 diabetic patients who were studied at baseline and after three years. A blood sample was taken for assessment of glycosylated haemoglobin (HbA1), lipid profile and a urine sample was taken for assessment of albumin/creatinine ratio, Neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid binding protein (L-FABP) and kidney injury molecule-1 (Kim-1) at baseline and after three years. Forty diabetic patients did renal Doppler at baseline & after three years.

RESULTS: HbA1, waist/hip ratio, albumin/creatinine ratio, lipid profile, NGAL, KIM-1, L-FABP and resistivity index (RI) were significantly increased in follow-up. Twenty patients (41.7%) showed progression to albuminuria. RI showed a significant increase in follow-up study. ROC curve showed that RI and NGAL had the highest sensitivity (100%), followed by L-FABP (90%) and lastly KIM-1 (63.6%) in the prediction of DN.

CONCLUSION: High RI, NGAL, KIM-1 & L-FABP can be considered as early markers of diabetic nephropathy in type 1 diabetics and are associated with its progression over time, independent of albuminuria.

Introduction

As a result of rising prevalence of type 1 diabetes, its complications including diabetic nephropathy (DN) affect more patients, but these complications rarely present clinically in childhood and adolescence. However, early functional and structural abnormalities may be present a few years after the onset of the disease [1-4]. Consequently, early sensitive markers that reflect the stage and course of diabetic nephropathy are needed to prevent progression to end-stage renal disease and dialysis as 1 of 3 patients with diabetic nephropathy develops end-stage renal disease (ESRD) [5] and the cumulative incidence of ESRD is 8%, 30 years after onset of type 1 diabetes (T1D) [6].

Our previous study [4] and many other recent studies [7-9] have shown that intrarenal resistivity index (RI), Neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid binding protein (L-FABP) and kidney injury molecule-1 (Kim-1) are promising early highly sensitive and highly specific (especially NGAL) markers for diagnosis of DN even in pre albuminuric stage because it has been shown that renal tubular damage can precede microalbuminuria and consequently allow early detection of DN, offering a good chance for delaying or even preventing progression to end-stage renal disease. However, the role of these early markers in the progression of diabetic nephropathy needs further evaluation.

Previous studies including our previous study [4] have shown that urinary levels of the tubular markers NGAL and KIM-1 [9, 10] and urinary L-FABP [11, 12] are increased in type 1 diabetic (T1D) patients, even before they develop signs of glomerular damage, i.e. micro- or macroalbuminuria. Also, u-NGAL is increased in type I diabetic patients compared to healthy controls, and increases with increasing levels of albuminuria [4, 10], so NGAL

represents an early biomarker of ‘normoalbuminuric’’ DN with a good sensitivity and specificity.

In the current work, we are aiming to explore and evaluate whether increased intrarenal resistivity index (RI) and increased different biomarkers of diabetic nephropathy (DN) are associated with clinical signs of DN and its progression over time i.e. predictors of progression thus representing important non-invasive tests to make precocious diagnosis of ‘‘normoalbuminuric" DN.

Patients and Methods

Patients

This longitudinal study included 48 type 1 diabetic patients among those attending to the endocrine clinic, National Research Centre, who were studied at baseline and after three years.

Inclusion criteria at baseline: Patients with duration of disease > 5 years, patients age > 14 and < 19 yrs old.

Exclusion criteria were as follows: Patients during acute diabetic complications e.g. diabetic ketoacidosis (DKA) or hypoglycemia, Patients suffering from cardiac diseases e.g. congenital, rheumatic heart, left ventricular dysfunction, Patients receiving drugs for cardiovascular disease.

Study design and protocol

It is a longitudinal study done after obtaining approval from the ethical committee of the National Research Centre, Cairo, Egypt. The registration number is 14058. It conforms to the provisions of the Declaration of Helsinki (as revised in Edinburgh 2000). Written informed consent was obtained from all patients, their parents and controls after a full discussion about the aim of the study. This study is a part of a project done at the National Research Centre in collaboration with Cairo University for evaluation of cardiac, vascular and endothelial function in adolescent type 1 diabetic patients.

All the studied patients were subjected to history taking including the age of patients, sex, the age of onset of diabetes, duration of diabetes, type and dose of insulin therapy, family history of diabetes. We asked about the presence of any symptoms of cardiac, renal, neurological affection or presence of any autonomic dysfunction. We also asked about the history of taking drugs other than insulin.

Patients were subjected, at baseline and after three years, for general, cardiac, chest and neurological examination.

Blood pressure was measured three times for patients after 5-minute rest in the sitting position on both upper limbs with the use of automatic manometer (Omron M4 Plus, Omron Health care Europe, Hoof drop, and Holland). The mean value of the second and the third measurement was calculated. The measurements taken on the dominant limb were analysed.

All patients underwent the following tests at baseline and after three years:

For cholesterol measurements, venous blood was sampled after 12 hr fast. Serum total cholesterol was determined by a commercial kit (Boehringer-Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was separated from the serum by precipitation of the other lipoproteins with a heparin/manganese procedure [13]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. The concentration of triglycerides was measured in a TechnoCon AutoAnalyzer II (TechnoCon Instruments, Tarrytown, New York).

Glycosylated haemoglobin (HbA1) was determined spectrophotometrically using commercially kit supplied by Stanbio, USA according to the method described by Trivelli et al. [14]. Glycosylated haemoglobin (HbA1) was done every three months, and the mean value was calculated from the four readings of the last year, at baseline and after three years.

Screening for microalbuminuria was assessed in fresh morning urine samples by measuring albumin/creatinine ratio by enzyme-linked immunosorbent assay (ELISA) kit provided by (Orgentec Diagnostika, Gmbh, Mainz, Germany) [15].

Freshly voided urine specimens were collected early in the morning from patients and controls. Urine samples were placed at 4°C and transported directly to the laboratory where they were centrifuged to remove sediment and frozen in aliquots at −80°C for further analysis. Urinary biomarkers levels were standardised to urine creatinine measured in the same spot urine and expressed related to mg Cr.

Urinary NGAL and KIM-1 were assessed by enzyme-linked immunosorbent assay (ELISA) using a kit from Quantikine; R & D Systems, Minneapolis; USA. L-FABP was measured with a commercially available ELISA kit according to manufacturer's protocol (Biovendor Laboratory Medicine, Brno, Czech Republic). The inter- and intraassay coefficients of variation for NGAL, KIM-1, and L-FABP were <10%. The measurements were made in duplicate and a blinded fashion.

Only 40 patients were subjected to renal colour duplex ultrasound scans at baseline & after three years, using 3-6 MHz convex array transducer (Toshiba, Xario ultrasound machine). Patients were scanned in the supine position. The transducer was

placed in longitudinal position just to the Lt. of the midline, recording colour flow & Doppler spectrum from the abdominal aorta where peak systolic velocity of the abdominal aorta was recorded. Then, the transducer was placed in transverse position just distal to the origin of superior mesenteric artery, to achieve transverse view of the aorta at the origins of both renal arteries where peak systolic velocity of both renal arteries was recorded, and renal artery stenosis was ruled out in all patients by tracing and examining different segments of both renal arteries form origin to renal hilum. Then, resistivity indices were recorded in the segmental, interlobar and arcuate arteries, on both sides.

Statistical Analysis

T-test for dependent and independent variables was used for analysis of data. McNemar test was also used. ROC curve was used for detection of cut-off value for detection of best sensitivity and specificity of NGAL, KIM-1, L-FABP and RI.

Results

This longitudinal study included 48 type 1 diabetic patients who were studied at baseline and after three years.

Table 1: Descriptive statistics of basal demographic, anthropometric and laboratory data of diabetic patients

Basal	Minimal	Maximum	Mean	SD
Age (yrs)	13.50	19.30	16.17	1.58
Duration of disease (yrs)	5.00	16.30	9.17	2.87
Insulin (U/kg)	0.77	2.03	1.38	0.32
Systolic blood pressure (mmHg)	100.00	160.00	119	13.64
Diastolic blood pressure (mmHg)	60.00	100.00	76.95	10.66
Waist circumference (cm)	69.00	103.50	82.76	9.12
Hip circumference (cm)	78.50	106.00	91.70	7.21
Waist/hip ratio	0.78	1.06	0.87	0.09
Waist/height ratio	0.42	0.65	0.51	0.06
BMI (SDS)	-1.10	2.70	1.24	0.89
BMI (Kg/m^2)	18.20	31.40	24.47	3.61
HbA1 (%)	5.90	15.00	8.98	1.66
Albumin/creatinine ratio (µg/g)	7.61	384.20	26.84	45.94
Cholesterol (mg/dl)	100.00	431.00	188.7	70.83
Triglycerides (mg/dl)	35.00	289.00	96.61	58.22
HDL-c (mg/dl)	21.00	136.00	51.68	22.99
LDL-c (mg/dl)	22.00	210.00	111.86	33.36
OxLDL (mg/dl)	8.70	37.10	18.35	7.16
Urinary NGAL (g/ml)	97.00	378.00	178.50	62.73
Urinary Kim-1 (ng/g creatinine)	21.00	216.00	70.69	51.37
Urinary L-FABP (µg/g creatinine)	1.50	58.60	15.41	26.14
Resistivity Index	0.55	0.74	0.60	0.04

BMI, Body mass index; HbA1, glycosylated haemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OxLDL, oxidised low-density lipoprotein; NGAL, neutrophil gelatinase associated lipocalin; L-FABP, liver-type fatty acid binding protein; Kim-1, urine levels of kidney injury molecule-1.

The studied group comprised of 26 females and 22 males, with a baseline mean age of 16.17 ± 1.58 years and duration of diabetes of 9.17 ± 2.87 years and a follow-up mean age of 19.46 ±1.52 years and duration of diabetes of 12 ± 3.59 years. All diabetic patients were on intensive insulin therapy regimen.

Table 2: Descriptive statistics of follow-up demographic, anthropometric and laboratory data of diabetic patients

Follow-up	Minimal	Maximum	Mean	SD
Age (yrs)	17.00	23.00	19.46	1.52
Duration of disease (yrs)	8.00	20.50	12.00	3.59
Onset of disease (yrs)	1.50	17.00	7.47	3.24
Systolic blood pressure (mmHg)	100.00	150.00	120	13.0
Diastolic blood pressure (mmHg)	50.00	100.00	80.49	8.86
Waist circumference (cm)	50.00	103.00	81.88	10.67
Hip circumference (cm)	78.00	113.00	94.56	8.59
HbA1 (%)	6.00	15.00	9.51	1.84
Albumin/ creatinine ratio (µg/g)	11.00	400.00	87.60	103.0
Cholesterol (mg/dl)	84.00	400.00	208.8	74.60
Triglycerides (mg/dl)	36.00	288.00	116.83	58.46
HDL-c (mg/dl)	30.00	130.00	50.91	19.66
LDL-c (mg/dl)	38.00	231.00	117.56	39.90
OxLDL (mg/dl)	1.30	7.80	43.93	15.13
Urinary NGAL (g/ml)	25.70	410.70	212.41	72.81
Urinary Kim-1 (ng/g creatinine)	0.10	46.30	145.08	90.24
Urinary L-FABP (µg/g creatinine)	2.00	120.00	20.19	28.96
Resistivity Index	0.60	0.74	0.66	0.03

BMI, Body mass index; HbA1, glycosylated haemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OxLDL, oxidised low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid binding protein; Kim-1, urine levels of kidney injury molecule-1.

Only 40 diabetic patients did renal Doppler, while all patients did all laboratory investigation, at baseline and after three years. Descriptive statistics of demographic, anthropometric and laboratory data (basal and after three years) of diabetic patients were shown in Tables 1 and 2. Comparison between demographic, laboratory data and resistivity index of patients at baseline and after three years, were shown in Table 3.

Table 3: Comparison between demographic, laboratory data and renal Doppler at the beginning and after three years follow-up in type 1 diabetic patients (n = 48)

Variables	Basal		Follow-up		P-value
	Mean	SD	Mean	SD	
Demographic data:					
Systolic blood pressure (mmHg)	119	13.64	120	13.0	0.7
Diastolic blood pressure (mmHg)	76.95	10.66	80.49	8.86	0.04
BMI (kg/m^2)	24.47	3.61	24.67	3.15	0.7
Waist/hip	0.87	0.09	0.90	0.07	0.02
Waist/height	0.51	0.06	0.50	0.07	0.3
Laboratory data:					
HbA1 (%)	8.98	1.66	9.51	1.84	0.003
Albumin/ creatinine ratio (µg/g)	26.84	45.94	87.60	103.0	0.007
Cholesterol (mg/dl)	188.7	70.83	208.8	74.60	0.002
Triglyceride (mg/dl)	96.61	58.22	116.83	58.46	0.0001
HDL-c (mg/dl)	51.68	22.99	50.91	19.66	0.8
LDL-c (mg/dl)	111.86	33.36	117.56	39.90	0.02
OxLDL(mg/dl)	18.35	7.16	43.93	15.13	0.0001
Urinary NGAL (g/ml)	178.50	62.73	212.41	72.81	0.002
Urinary Kim-1 (ng/g creatinine)	70.69	51.37	145.08	90.24	0.0001
Urinary L-FABP (µg/g creatinine)	15.41	26.14	20.19	28.96	0.01
Resistivity Index	0.60	0.04	0.66	0.03	0.0001

t-Test for independent variables; BMI, Body mass index; HbA1, glycosylated haemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OxLDL, Oxidised low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid binding protein; Kim-1, urine levels of kidney injury molecule-1. The bold values in the table indicate significance.

HbA1, diastolic blood pressure, waist/hip ratio, albumin/ creatinine ratio, lipid profile, NGAL, KIM-1, L-FABP and RI were significantly increased in follow-up. Twenty patients (41.7%) showed progression to albuminuria while the remaining 28 patients (58.3%) showed stationary or regressed albuminuria. The progressed group showed significantly high basal NGAL (p = 0.01), basal KIM-1 (p = 0.01) & basal L-FABP (p = 0.04) with high basal RI but not statistically significant (p = 0.09) (Table 4).

Table 4: Comparison between basal NEGAL, KIM-1, L-FABP and resistivity index about microalbuminuria

Variables	Microalbuminuria				P-value
	Stationary or regressed N = 28		Progressed N = 20		
	Mean	SD	Mean	SD	
Urinary NGAL (g/ml)	155.20	47.85	199.04	69.14	0.01
Urinary Kim-1 (ng/g creatinine)	49.25	26.38	83.21	53.72	0.01
Urinary L-FABP (µg/g creatinine)	6.26	3.65	10.70	10.58	0.04
Resistivity Index	0.59	0.02	0.61	0.04	0.09

t-Test for independent variables. NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid binding protein; Kim-1, urine levels of kidney injury molecule-1. The bold values in the table indicate significance.

Basal NGAL, KIM-1, L-FABP & R.I. were high in diabetic patients who progressed to albuminuria and noted in 80%, 55%, 45% & 30% respectively at baseline (Table 5).

Table 5: Frequency distribution of basal level of NEGAL, KIM-1, L-FAB and resistivity index in diabetic patients with progressive albuminuria (N =20)

Basal	N	%
NGAL:		
Normal	4	20
Abnormal	16	80
KIM-1:		
Normal	9	45
Abnormal	11	55
L-FABP		
Normal	11	55
Abnormal	9	45
Resistivity Index:		
Normal	14	70
Abnormal	6	30

NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid binding protein; Kim-1, urine levels of kidney injury molecule-1.

RI showed a significant increase in follow-up study where R.I. was abnormally increased in 9 patients (22.5%) at baseline while increased in 33 patients (82.5%) in the follow-up study after three years (P value=0.0001) (Table 6).

Table 6: Comparison between resistivity index at the beginning and after three years follow-up (N =40)

Variables	Follow-up				P-value
	Normal		Abnormal		
Basal	N	%	N	%	
Normal (N = 31)	5	16.1	26	83.9	0.0001
Abnormal (N = 9)	2	22.2	7	77.8	

McNamara test was used for analysis of data. What abnormal resistivity index after three years follow-up was significantly higher in diabetic patients. The bold value in the table indicates significance.

ROC curve showed that RI and NGAL had the highest sensitivity (100%), followed by L-FABP (90%) and lastly was KIM-1 (63.6%) in the prediction of DN (Table 7).

Table 7: ROC curve of basal resistivity index, NEGAL, KIM-1 and L-FAB of diabetic of patients

Variables	RI	NGAL	KIM-1	L-FABP
Cut off	0.575	>94	>45	> 10.3
Area under the curve	0.587	0.645	0.606	0.941
SE	0.115	0.096	0.097	0.050
95% C.I.	0.434 – 0.728	0.527 – 0.752	0.487 – 0.716	0.862 – 0.982
Sensitivity	100	100	63.6	90.0
Specificity	28.2	32.3	61.5	87.7
PPV	1.39	1.48	1.65	6.65
NPV	0	0	0.59	0.21

PPV = Positive predictive value; NPV = Negative predictive value; C.I. = Confidence interval; NGAL = neutrophil gelatinase associated lipocalin; L-FABP = liver-type fatty acid binding protein; Kim-1= urine levels of kidney injury molecule-1; RI = resistivity index; RT = resistivity index.

Discussion

Early management of DN is very important to prevent progression to end-stage renal disease and dialysis, and the current commonly used parameter to diagnose DN is microalbuminuria which is a non-optimal biomarker with low sensitivity and specificity, so there is a necessity to explore and evaluate whether increased intrarenal resistivity index (RI) and increased different biomarkers, like NGAL, KIM-1 and L-FABP are associated with clinical signs of DN and its progression over time i.e. predictors of progression.

In our previous cross-sectional study [4], we have found that R.I., NGAL, L-FABP & KIM-1 are increased in type 1 diabetic patients compared to normal controls, and increase with increasing levels of albuminuria. Being a cross-sectional study, it does not describe the time perspective or causality, so these findings inspired us to look at these markers in a longitudinal study.

The current longitudinal study revealed significantly increased HbA1, diastolic blood pressure, waist/hip ratio, albumin/ creatinine ratio, lipid profile, NGAL, KIM-1, L-FABP and RI in the follow up after three years and twenty patients (41.7%) progressed to albuminuria while the remaining 28 patients (58.3%) showed stationary or regressed albuminuria.

The progressed group showed increased basal RI but not statistically significant (p = 0.09) as R.I. was increased in diabetic patients who progressed to albuminuria in 30% at baseline. RI showed a significant increase in follow-up a study where R.I. was abnormally increased in 9 patients (22.5%) at baseline while increased in 33 patients (82.5%) in the follow-up study after three years (P value = 0.0001). ROC curve revealed that RI showed a sensitivity of 100% in the prediction of DN. Our results are in agreement with the results seen in type 1 diabetic patients of many previous studies that demonstrated increased resistivity indices early in the course of DN in the pre albuminuric stage [4, 16, 17]. Also, Masulli et al. [18] reported similar results but in type 2 diabetic patients as they found that high RI is associated with features of DN and its progression over time, independent of albuminuria.

In our study, the progressed group showed significantly high basal NGAL (p = 0.01), basal KIM-1 (p = 0.01) & basal L-FABP (p = 0.04) where basal NGAL, KIM-1, & L-FABP were high in diabetic patients who progressed to albuminuria in 80%, 55% & 45%, respectively at baseline. NGAL, KIM-1 & L-FABP showed significant increase in follow-up (P values = 0.002, 0.0001, and 0.01 respectively). ROC curve revealed that NGAL showed a sensitivity of 100%, followed by L-FABP (90%) and lastly is KIM-1 (63.6%) in the prediction of DN. Our results are comparable with the previous studies on diabetic

patients that found that the levels of NGAL, KIM-1 and L-FABP are increased in type 1 diabetic patients, even before they develop albuminuria and also found that their levels were increased with increasing levels of albuminuria [4, 9-12, 19]. We found that NGAL has the highest sensitivity (100%) in the prediction of DN in line with the previous study that found a positive correlation between uNGAL and albuminuria and concluded that NGAL measurement could be useful for the evaluation of early renal involvement in the course of diabetes [19].

We conclude that resistivity indices and renal tubular biomarkers are increased before the appearance of pathological albuminuria, which is the earlier measurable sign of renal diabetic involvement, supporting the hypothesis of a tubular phase of DN preceding the glomerular phase and consequently, the increase in R.I. and renal tubular biomarkers' values could express the degree of subclinical tubular impairment preceding the classic glomerular signs. R.I. and these new tubular biomarkers offer an advantage to urinary albumin on early detection of DN. We recommend these non-invasive tests for monitoring type 1 diabetic patients to predict those at risk of diabetic nephropathy as this longitudinal study design allow us to conclude that RI and tubular biomarkers precede the development of diabetic nephropathy and microalbuminuria.

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