Color Doppler Sonographic Dynamic Tissue Perfusion Measurement Demonstrates Significantly Reduced Cortical Perfusion in Children with Diabetes Mellitus Type 1 without Microalbuminuria and Apparently Healthy Kidneys

Ultraschall in Med 2014; 35: 445–450

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Rüdigerstraße 14
70469 Stuttgart
ISSN 0172-4614

Color Doppler Sonographic Dynamic Tissue Perfusion Measurement Demonstrates Significantly Reduced Cortical Perfusion in Children with Diabetes Mellitus Type 1 without Microalbuminuria and Apparently Healthy Kidneys

Nachweis einer signifikanten kortikalen Perfusionsminderung in scheinbar gesunden Nieren von Kindern mit Diabetes mellitus Typ 1 ohne Mikroalbuminurie mithilfe der dynamischen farbdopplersonografischen Gewebsperfusionsmessung

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Key words
- ultrasound color Doppler
- dynamic tissue perfusion measurement
- kidney
- diabetes mellitus

Abstract

Motivation: With respect to the devastating consequences of the increasing prevalence of diabetes mellitus, the main reason for end stage renal disease and dialysis in industrialized countries, and the very limited diagnostic and therapeutic possibilities to predict, monitor and prevent diabetic nephropathy (DN), new concepts for early recognition and quantification of the prevailing microvascular changes in DN are urgently needed.

Materials and Methods: We present the first study of renal cortical tissue perfusion measurement by means of standardized color Doppler sonographic videos evaluated with the PixelFlux software 1 for Dynamic Tissue Perfusion Measurement (DTPM) in 92 patients with DM1 without MA compared to 71 healthy probands.

Results: DTPM reveals a highly significant diminution of cortical perfusion in patients with DM1 compared to healthy probands by 31 %, most pronounced in the distal hemicortex (reduction by 50 %) compared to 21 % within the proximal hemicortex.

Conclusion: Thus, DTPM offers a novel means of numerically describing the state of the renal microvasculature in DM in a patient-friendly, non-invasive, non-ionizing manner.

Zusammenfassung

Motivation: Die schwerwiegenden Folgen der zunehmenden Prävalenz des Diabetes mellitus, der Hauptursache terminalen Nierenversagens in den Industrieländern und die noch sehr begrenzten Möglichkeiten, die diabetische Nephropathie (DN) vorherzusehen, zu überwachen und zu verhindern verlangen nach neuen Konzepten der Früherkennung und Quantifizierung mikrovaskulärer Veränderungen.

Material und Methoden: Wir legen die erste Untersuchung zur standardisierten dynamischen farbdopplersonografischen Gewebsperfusionsmessung (DTPM) des renalen Kortex mit der PixelFlux-Software bei 92 Kindern mit Diabetes mellitus Typ 1 (DM1) ohne Mikroalbuminurie (MA) im Vergleich zu 71 gesunden Probanden vor.

Ergebnisse: Dabei fanden wir eine signifikante Perfusionsminderung bei Patienten mit DM1 im Vergleich zu Gesunden um 31 %. Sie war im distalen Hemikortex mit 50 % stärker als im proximalen Hemikortex (21 %) ausgeprägt.

Schlussfolgerung: Mit der DTPM kann die Schädigung kleinster renalen Gefäße bei DM patientenschonend quantifiziert werden.

received 9.8.2013
accepted 20.12.2013

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DOI http://dx.doi.org/10.1055/s-0034-1365909
Published online: February 20, 2014
Ultraschall in Med 2014; 35: 445–450 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0172-4614

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Introduction

Diabetes mellitus (DM) is a major individual and socioeconomic health burden due to damaging of the microvasculature of several organs, preferentially the kidney and eye. Reduced tissue perfusion paves the way to organ failure. Renal damage is suspected as soon as microalbuminuria (MA) occurs. MA is often regarded as a valuable predictor of ensuing diabetic nephropathy. Nevertheless, the relevance of MA for the development of end stage renal disease (ESRD) was questioned [1 – 3]. A low rate of microalbinuric patients were found to convert to progressive diabetic nephropathy (DN) [4, 5] and parameters closer to the pathophysiologic processes leading to ESRD are needed [6, 7]. In diabetes mellitus type 1 (DM1), about one-third of patients [5] develop MA. Latency from clinical onset of diabetes to first signs of diabetic nephropathy in DM1 was 10 years on average [5]. Thus, reliance on MA to expect renal DN to develop or to remain absent is not useful. Therefore, we investigated a novel approach to describe changes of the renal micro-

n = 92	height [cm]	weight [kg]	BMI [kg/m²]	BSA [m²]	age [years]
mean	155.26	50.93	20.35	1.47	12.93
standard error of the mean	2.05	1.79	0.35	0.04	0.36
standard deviation	19.66	17.19	3.34	0.34	3.49
minimum	99.80	16.20	14.15	0.67	4.02
maximum	188.40	85.80	29.90	2.09	17.99

Table 1 Physical data from 92 children and adolescents with DM1 without MA.

vasculature in DM1. We applied the sonographic technique of dynamic tissue perfusion measurement (DTPM) [8 – 11] in a cross-sectional study to compare cortical perfusion in kidneys from patients with DM1 and normal age-matched controls.

Materials and Methods

Patients

From April to September 2010, we investigated 92 children and adolescents with DM1 (**Table 1**) without MA. Their HbA1 values and the duration of diabetes are given in **Table 2**. In addition, we investigated 71 healthy children (**Table 3**) without anamnestic, actual or familial signs of renal disease or DM and normal renal ultrasound findings (size, echogenicity, parenchymal thickness, cortico-medullary differentiation). The patients were recruited from our pediatric diabetes outpatient department and were enrolled after informed consent of both parents and patients. Healthy children were identified in a sonographic outpatient department or from inpatients of our hospital. Both groups stemmed from the same local population and pediatric age group with comparable height, weight, BSA and BMI. No stratification as to age subgroups or gender was made. Paraclinical data (HbA1c) were drawn from routine surveillance protocols of diabetic patients.

Table 2 HbA1 values and duration of DM1 in studied patients.

HBA1c (%) Kolmogorov-Smirnov test p = 0.286			duration of DM1 (years) Kolmogorov-Smirnov test p = 0.449
n		92	92
mean		8.409	5.847
standard error of the mean		0.1459	0.3590
median		8.100	5.377
standard deviation		1.3993	3.4434
minimum		5.4	0
maximum		14.1	16.4
percentile	3	6.300	0.847
	10	6.630	1.985
	20	7.400	2.756
	25	7.425	2.923
	30	7.700	3.200
	40	7.900	4.762
	50	8.100	5.377
	60	8.500	6.189
	70	9.100	7.266
	75	9.300	7.619
	80	9.440	8.608
	90	10.140	10.600
	97	11.563	13.493

Methods

Color Doppler ultrasound

All ultrasound examinations were always done with the patient in a prone position by the same investigator (TS) with more than 25 years ultrasound experience. A Siemens ACUSON S2000 ultrasound machine equipped with a curved array transducer offering a frequency range from 4 to 1 MHz in B-mode and 3.5 MHz in color Doppler mode was used.

Dynamic tissue perfusion measurement (DTPM)

Standardized recording

The color Doppler ultrasound examination was performed with a fixed algorithm and predefined machine settings (fixed preset) in all patients and healthy probands. Standardized recordings of color Doppler sonographic videos in DICOM format were transferred to a personal computer where DTPM was carried out with the PixelFlux software (Chameleon-Software, Germany) [12].

The central part of the kidney in a longitudinal section was enlarged so that the central segment and parts of its neighboring segments were to be seen clearly and as large as possible.

The central interlobar artery was running straight towards the transducer with its arcuate arteries branching symmetrically to both sides (**Fig. 1**). The outer surface of the kidney could be discriminated from the surrounding fat (high echogenicity) by a discrete demarcation border. With a fixed color Doppler sonographic preset (transducer 4C1; color frequency 3.5 MHz, harmonic B-mode frequency 4.5 MHz, maximum color coded velocity 11 cm/s, further manufacturer-specific settings: low flow [meaning: entire machine setting adjusted for optimal detection of low velocity flow phenomena], D1 [meaning: color persistence at 1, range 0 – 4], ST3 [balance between spatial and time resolution at 3 from 1 to 5], PRF 977 [pulse repetition frequency 977 Hz], F2 [wall filter at 2, range 0 – 3], S1 [smoothing of the image at 1, range 0 – 3], Pr4 [color priority of the image at 4, range 0 – 4]) a video sequence of 2 seconds was recorded in breath holding technique. Each video contained at least one full heart cycle. If necessary, the color frequency and maximum flow velocity were adjusted to the patient's size. The PixelFlux software takes these changes into account and calculates flow velocities within the ROI according to the adjusted flow velocities.

Perfusion measurement

All videos were automatically calibrated for distances and color hues by PXFX. The region of interest (ROI) was defined as a parallelogram encompassing one full cortical segment from the outer borders of the medullary pyramids (MP) to the renal capsule and laterally from the center of one MP to the center of the neighboring MP (**Fig. 1**). This means that the ROI had a different size in different patients. Its size corresponded to the individual anatomic landmarks, renal surface and medullary pyramids, which were spaced less in younger than in older (larger) children. In this way it was assured that the proximal 50 % of the renal cortex was always compared to the individual's distal

n = 71	height [cm]	weight [kg]	BMI [kg/m²]	BSA [m²]	age [years]
mean	132.30	32.37	16.96	1.08	8.97
standard error of the mean	3.35	1.93	0.41	0.05	0.56
standard deviation	28.45	16.42	3.45	0.39	4.71
minimum	44.00	1.90	9.81	0.15	4.02
maximum	187.00	73.50	28.01	1.88	18.00

Table 3 Physical data from 71 healthy children.

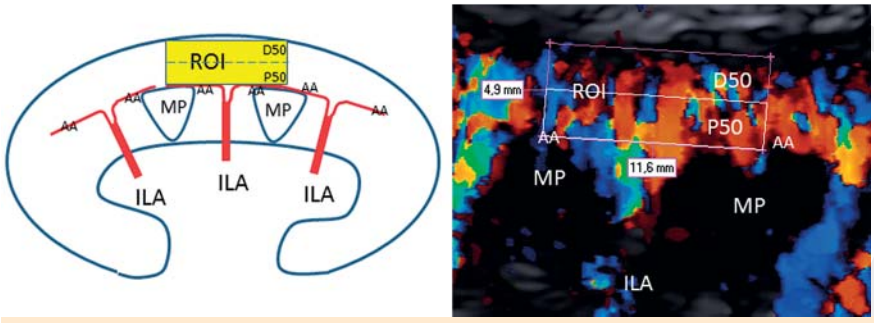

Fig. 1 Drawing of the relevant renal landmarks (MP: medullary pyramids; AA: Arcuate areries; ILA: interlobar arteries) for definition of the region of interest (left) and color Doppler sonogram with the same landmarks and ROI as well as proximal and distal 50 % layers of the ROI (right).

Abb. 1 Links: Skizze der anatomischen Strukturen (MP: Markkegel, AA: Aa. Arcuatae, ILA: Aa. Interlobares) zur Positionierung der Untersuchungsregion (ROI). Rechts: ROI mit horizontalen sub-ROIs (P50: proximale 50 % des, D50: distale 50 %) im Farbdopplerbild mit zur Skizze korrespondierenden Strukturen.

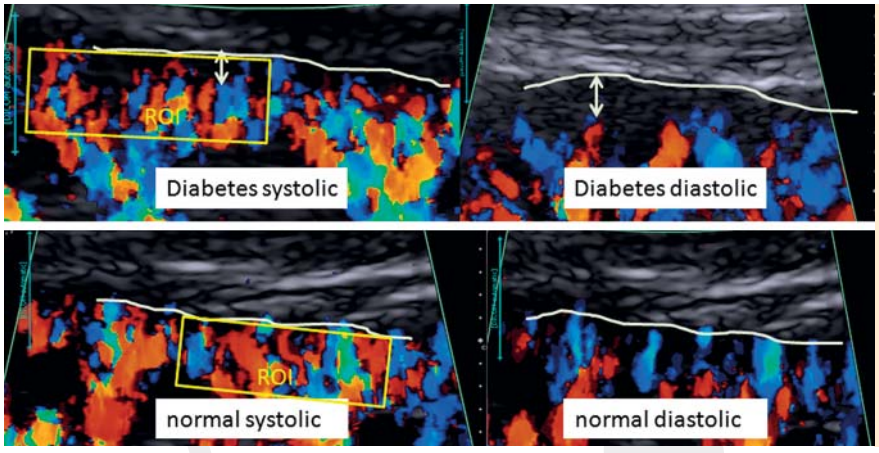

Fig. 2 Color Doppler still images from a kidney of a child with DM1 (upper line) and a normal kidney from a healthy child (lower line). The white lines mark the renal capsule. Double arrows indicate the widened hypovascularized subcapsular rim in DM1. See the positioning of the ROI (compare to **Fig. 1**).

Abb. 2 Farbdopplerbilder der Niere eines Kindes mit DM1 (oben) und der Niere eines gesunden Kindes (unten) in Systole (links) und Diastole (rechts). Die Nierenkapsel ist weiß markiert. Doppelpfeile markieren den bei der diabetischen Niere erweiterten gefäßverarmten subkapsulären Saum. Festlegung der ROI wie in **Abb. 1**.

50 % of the renal cortical thickness. Care was taken to observe the watersheds of blood flow between two neighboring cortical segments as lateral borders of the ROI (**Fig. 2**). The ROI was horizontally sliced in equal-sized sub-ROIs each encompassing the proximal or distal 50 % (P50 and D50) and horizontal slices with a thickness of 10 % of the cortex. Perfusion measurement was then carried out automatically by PXFX.

Perfusion intensity (PI) is calculated as:

$PI\ [cm/s] = v\ [cm/s]^{*}\ A\ [cm^2]/A_{ROI}\ [cm^2]$

- v: mean velocity value of all pixels of the ROI at a certain time
- A: means are occupied by all pixels of the ROI at a certain time
- A_{ROI}: area of the ROI

All measurements of v and A were done image by image for the entire video. PXFX then recognized complete heart cycles within the video and calculated the mean values for v and A for complete heart cycles only. PI thus combines all relevant data that determine blood flow intensity per heart cycle.

Statistics

For comparisons between groups of patients, the Mann-Whitney-U-test was applied. Correlations were evaluated by the Spearman rank correlation or Pearson's correlation if suitable. The Kolmogorov-Smirnov test was applied to test for normal distribution. Probabilities less than 5 % were regarded as statistically significant.

Results

Duration of diabetes mellitus and HBA1 level

HbA1c values and duration of DM1 in diabetic patients were normally distributed (data shown in **Table 2**).

Cortical tissue perfusion intensity

We found a highly significant reduction of flow intensity in DM1 patients compared to the healthy group. The difference was more

	full cortex		distal cortex		proximal cortex	
	healthy	DM1	healthy	DM1	healthy	DM1
n	71	92	71	92	71	92
minimum [cm/s]	0.10	0.11	0.02	0.16	0.18	0.23
maximum [cm/s]	3.64	2.57	3.11	1.60	5.20	3.72
mean [cm/s]	1.67	1.16	1.11	0.55	2.26	1.78
standard deviation	0.94	0.54	0.77	0.41	1.21	0.75
p (Mann – Whitney – U-test)	0.001		< 0.001		0.015	

Table 4 Comparison of renal cortical perfusion intensities in 92 patients with DM1 and 71 healthy children within the full cortical width distal 50 % and proximal 50 % of the renal cortex.

Table 5 Perfusion gradients (percentage of perfusion loss from central to peripheral cortical layers) in patients with DM1.

group		perfusion gradient d50 / p50 [1]	perfusion gradient p9 / p2 [1]
healthy	median	– 56 %	– 74 %
	minimum	– 94 %	– 100 %
	maximum	10 %	– 23 %
	SD [2]	0.212	0.207
	n	71	71
diabetic patients	median	– 72 %	– 90 %
	minimum	– 97 %	– 100 %
	maximum	– 39 %	– 43 %
	SD [2]	0.145	0.118
	n	92	92
asymptotic significance (2-sided)	p	< 0,001	< 0,001

[1] Negative values describe a reduction in the perfusion intensities from the proximal to peripheral cortex;
[2] SD: standard deviation

Table 6 No significant correlation of cortical perfusion intensity in the group of healthy children to age, height, weight, BMI and BSA.

perfusion intensity [cm/s] in ROI d50	correlated to	age [a]	height [cm]	weight [kg]	BMI [kg/m²]	BSA [m²]
Pearson's correlation coefficient		– 0.083	– 0.035	– 0.078	– 0.073	– 0.064
p		0.329	0.679	0.356	0.388	0.447
n		142	142	142	142	142

pronounced in the distal cortex (ROI D50) compared to the proximal one (ROI P50) (**Table 4**). Perfusion gradients from the proximal to distal cortex were significantly different in DM1 patients compared to healthy children (**Table 5**). These results are not confounded by age, weight, height, BMI or BSA (**Table 6**).

Discussion

DN is the main cause of the increased morbidity and mortality in insulin-dependent diabetics [13] and an important risk of death especially in younger adult patients [14]. In a large follow-up study of DM1 patients, 45 % developed cumulatively DN after 40 years of DM [15]. Genetic factors determining DM progression towards DN are not yet defined or might be subject to epigenetic influences [16]. Thus, today the occurrence of MA is still the only clinically relevant criterion to support the suspicion of a developing DN. Research is focusing on details of the developing loss of urinary protein. Direct measures of vascular changes are not feasible in most cases, since they would require invasive techniques such as biopsies. Urinary proteome analysis in DN was helpful to classify the stage of DN, may be indicative of beneficial effects of an antihypertensive treatment in these patients [17] and even may allow differential diagnosis [18].

Nevertheless, proteomics are a trace of the morphological changes (accumulation of extracellular matrix and mesangial proliferation in the glomerulus) already present [19]. It would be worthwhile to detect the antecedents of manifest histological changes and to focus on the structure that is abundant in kidneys and crucial to their function and non-functioning – the renal microvasculature. Color Doppler seems especially suitable to fulfill this task.

Fig. 2 emblematically illustrates subcapsular microvessel damage in a kidney from a child with DM1 (upper line) compared to a normal kidney from a healthy child. In DM1 the hypovascularized subcapsular rim can be clearly seen (double arrows), especially in diastole. This slowly developing damage cannot be evaluated with other ultrasound techniques preceding DTPM. DTPM now quantifies the distal (and invisible for the naked eye) also the proximal cortical hypoperfusion in DM1.

Renal Doppler investigations traditionally refer to the Resistance Index (RI) [20, 21]. Its manifold limitations stem from its restricted database: the maximum systolic and the end-diastolic flow velocity of a few (mostly 1 to 3) intrarenal arteries are measured to calculate the $RI = v_{sys} - v_{dia} / v_{sys}$. The main disadvantage is that the RI does not take into account the loss of microvessels. Since the measurements are guided by the search for a color signal to direct the Doppler instrument, only existing vessels are evaluated. In vanishing vasculature there is no possibility to reflect the paucity of vessels by the RI [22]. Moreover, the perfused area is not reflected by the RI. Therefore, the RI is a marker of changing punctual velocities in a few intrarenal vessels. The volume of blood running through a tissue is only determined by the mean flow velocities in all vessels of an ROI and by their mean perfused area. No traditional Doppler technique takes both parameters into account. Here lies the advantage of DTPM which has been proven to be superior to RI to describe histological changes in renal transplants [23]. DTPM also reflects renal cortical fibrosis and can describe tissue oxygenation [24]. RI is subject to many extrarenal influences which further limit its value [25 – 28].

Color Doppler sonographic dynamic tissue perfusion measurement (DTPM) is capable of measuring tissue-specific perfusion intensities thus substantially expanding the reach of conventional Doppler techniques. For the first time a simple, bedside technique allows quantitative evaluation of renal microvessels in a structured manner, from the proximal to peripheral cortex, thus

connecting perfusion data to different branching levels of the vascular tree and histological changes in kidneys [8 – 11, 29]. Compared to traditional RI measurements that detect significant differences only between normalbuminuric and macroalbuminuric patients with DM2 [30], we could already detect microvascular damage in children before MA appeared.

The limits of this study are its single-center design, its restriction to young patients exclusively with DM1 and the difficulty to compare our results with those from other groups directly. In order to compare DTPM results directly among different centers, the same ultrasound machines and the same machine presets have to be used. The machine we used is widely but not universally used. Two solutions to this drawback are possible: 1. to calibrate the machine settings with a flow phantom: we developed a flow phantom and could demonstrate a highly significant correlation of PXFX measurements with a direct flow volume measurement [31] and with laser Doppler measurements (data not shown); 2. to use a ratio of distal to proximal perfusion (**Table 5**). The perfusion ratio then is comparable between different ultrasound machines since both proximal and distal cortical perfusion is recorded under identical conditions. The loss of the tiniest microvessels, which prevail in the subcapsular rim, is reflected by the perfusion ratio of proximal and distal cortical perfusion. This ratio is independent of the ultrasound system since in an individual patient both regions are recorded under identical conditions. The comparison of healthy and diabetic children demonstrated that the perfusion gradients, i. e. the dominant loss of microvessels, were significantly higher in diabetic children (**Table 5**). The perfusion drop from the proximal to distal 50 % of the cortex (d50 / p50 gradient) was 72 % in DM1 patients compared to 56 % in healthy children. Equally significant was the decline from the second (P2) to the ninth (P9) 10 %-layer (90 % vs. 74 %) (**Table 5**). We recommend the technically more robust d50 / p50 gradient for further studies since the very thin 10 % layers will be more affected by slight malposition of the ROI.

We examined children and adolescents with DM1 and no detectable renal changes in B-mode ultrasound imaging and urine analysis. None of the patients was microalbuminuric. Nevertheless, DTPM was able to find a striking diminution of perfusion intensity in these apparently normal kidneys. It was demonstrated before that the distal cortical layers are less perfused that the proximal ones. These encompass the arcuate vessels and the stem vessels of the interlobular ones. With their ascent to the cortical periphery they lose perfusion [8] due to vessels that branch off to feed midcortical glomeruli. They taper until they have distributed all the blood that has entered their stem at the base of the ROI. Incipient microvessel diameter reduction must affect the top of the vascular tree first – as shown in renal transplants [9, 10] and chronic renal insufficiency [32]. If the documented perfusion loss is already a sign of morphological changes within the interlobular arteries themselves, or caused by vessel constriction, or if the flow reduction is simply reflecting the decreasing acceptance of blood volume by shrinking glomerular passthrough, is an open question. With respect to the patients' young age and short duration of DM1, it seems likely that we observe here a functional adaptation. Animal models might clarify the morphological or functional correlate of this surprisingly significant perfusion loss. Rarely, sequential biopsies in DM1-patients are carried out. After all, in DM1 patients with a disease duration of 17 +/– 7 years morphological changes were found at first biopsy and were more developed after 5 years (mesangial fractional volume increased, arteriolar hyalinosis lesions progressed) [33]. These morphological changes are reversible in DM1 after 10 years of normoglycemia following pancreas transplantation and returned to normal values in some of the patients [34]. In light of these findings, very early detection of vascular glomerular and interstitial damage, thickening and hyalinization of intrarenal vasculature [35] is urgently needed since microvascular destruction is central to the progression of DN [36].

Our observations might offer a novel perspective on the initiation and progression of these microvascular changes in DN.

Abbreviations

BMI	Body mass index
BSA	Body surface area
DM	Diabetes mellitus
DM1	Diabetes mellitus type 1
DN	Diabetic nephropathy
DTPM	Dynamic tissue perfusion measurement
ESRD	End stage renal disease
MA	Microalbuminuria
MP	Medullary pyramids
PI	Perfusion intensity
PXFX	PixelFlux software
ROI	Region of interest
P50	Proximal 50 % of the ROI encompassing the cortical thickness from the outer edge of the medullary pyramids to the renal surface (**Fig. 1**)
D50	Distal 50 % of the ROI encompassing the cortical thickness from the outer edge of the medullary pyramids to the renal surface (**Fig. 1**)
P2	Second proximal layer of the ROI sliced into 10 equal horizontal layers
P9	Second to last proximal layer of the ROI sliced into 10 equal horizontal layers
TPI	Tissue pulsatility index
TRI	Tissue resistance index

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