SENSITIVITY AND SPECIFICITY OF MICRAL TEST AS A SCREENING METHOD FOR DIABETIC NEPHROPATHY IN TYPE 1 DIABETES PATIENTS

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REZUMAT

Introducere: Nefropatia diabetică reprezintă principala cauză de insuficiență renală cronică, iar diagnosticul ei precoce permite intervenția terapeutică promptă în scopul încetinirii evoluției spre faze mai avansate. Metodele actuale de diagnostic a nefropatiei diabetice, dozarea albuminuriei prin radioimunoanaliză (RIA), ELISA sau metoda imunoturbidimetrică (ITM), sunt laborioase și necesită dotare corespunzătoare, putând fi înlocuite cu teste de tipul testului Micral, opțiune valabilă mai ales pentru screeningul nefropatiei diabetice. Evaluarea unor astfel de teste necesită determinarea sensibilității și a specificității comparativ cu o metodă etalon. **Obiective:** Studiul are drept scop evaluarea sensibilității și specificității testului semicantitativ Micral în screening-ul nefropatiei diabetice la copiii și tinerii cu diabet zaharat (DZ) tip 1, comparativ cu dozarea cantitativă a microalbuminuriei prin metoda ITM. **Pacienți și metode:** Studiul a fost realizat pe un lot de 310 copii și tineri cu DZ tip 1 (vârstă între 12 și 30 ani) la care albuminuria a fost dozată prin două metode: metoda imunoturbidimetrică (ITM), ca metodă etalon (VN < 30 mg/24 h) și testul semicantitativ Micral (VN < 20mg/l) ca metodă supusă evaluării. Dozarea albuminuriei s-a făcut prin ambele metode, la 205 pacienți, din urina colectată în decurs de 24 ore, iar la 105 pacienți din prima urină matinală. S-a calculat sensibilitatea, specificitatea, valoarea predictivă pozitivă (VPP) și valoarea predictivă negativă (VPN) corespunzătoare testului Micral, folosind atât urina colectată în 24 ore cât și prima urină de dimineață. **Rezultate:** Folosind pentru screening urina de 24 ore, sensibilitatea și specificitatea testului Micral au fost de 94,6% și respectiv de 82,2%. VPP a fost 57,9%, iar VPN de 98,8%. Folosind un eșantion din prima urină de dimineață, testul Micral a avut o sensibilitate de 90,9% și o specificitate de 90,6 %. VPP în acest caz a fost 71,4%, iar VPN 97,4%. **Discuții:** Testul Micral a generat un număr crescut de rezultate fals pozitive, ceea ce se reflectă printr-o VPP scăzută, mai accentuată în cazul urinei de 24 ore. Rezultatele fals pozitive, mai puține în cazul utilizării unui eșantion din prima urină de dimineață, explică sensibilitatea semnificativ mai mare, comparativ cu utilizarea urinei de 24 ore. VPN se menține ridicată în cazul ambelor tipuri de eșantioane de urină. **Concluzii:** Sensibilitatea și specificitatea testului Micral îl recomandă ca metodă de screening a microalbuminuriei, în special de excludere a nefropatiei (VPN ridicată), dar, din cauza frecventelor rezultate fals pozitive, confirmarea diagnosticului de nefropatie diabetică necesită repetarea testării sau utilizarea unei metode cantitative.

Cuvinte cheie: nefropatie diabetică, microalbuminurie, screening, test Micral

ABSTRACT

Background: Diabetic nephropathy is the main cause of end-stage renal disease. Its early diagnosis allows for prompt therapeutic interventions that aim to slow disease progression towards more advanced stages. The current diagnostic procedure for diabetic nephropathy is represented by measurement of urinary albumin excretion using radioimmunoassay (RIA), ELISA, or immunoturbidimetric method (ITM). However, these techniques are laborious and require adequate equipment; thus simpler methods, such as Micral testing can be used instead, especially as a screening tool. This latter method needs to be evaluated for sensitivity as well as for specificity. **Objectives:** The goal of this study is to assess the sensitivity and specificity of semiquantitative Micral testing in the screening of DN in children and young with type 1 diabetes mellitus (DM), compared to a quantitative method for micralbuminuria, represented by ITM. **Patients and methods:** The study enrolled 310 subjects with type 1 DM, aged between 12 and 30 years, in whom albuminuria was measured using two assays: the immunoturbidimetric method (ITM), as the gold standard (normal range: î 30 mg/24 hs) and the semiquantitative Micral test (normal range î 20 mg/l), as the investigated method. Albuminuria was measured using both methods in all patients, using a 24h-urine sample in 205 patients, and first-void morning urine in 105 patients. Thereafter, sensitivity, specificity, positive as well as negative predictive value (PPV and NPV) were computed for Micral testing, using values measured both in 24h-urine and in first-void morning urine. **Results:** When 24h urine was used, Micral assay showed a sensitivity of 94.6% and a specificity of 82.2%, with a PPV of 57.9% and a NPV of 98.8%. When first-void urine samples were used, sensitivity and specificity of Micral were 90.9 and 90.6%, respectively, with a PPV of 71.4% and a NPV of 97.4%. **Discussions:** Micral testing generated a large number of false-positive results, that reflected into a lower PPV;, PPV decreased further when a 24h-urine sample was used. False-positive results were less encountered for first-void morning urine, and thus in this situation the sensitivity was higher. NPV is high for both types of urine samples. **Conclusions:** The sensitivity and specificity of Micral recommend it as a good screening method for microalbuminuria, especially for exclusion of diabetic nephropathy as the NPV is high. However, because of the large number of false-positive results, the diagnosis of diabetic nephropathy needs to be confirmed by repeated testing or by a quantitative method.

Key Words: diabetic nephropathy, microalbuminuria, screening, Micral testing

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BACKGROUND

Microalbuminuria, defined by an urinary albumin excretion rate between 30 and 300 mg/24 h, represents a predictive factor for progression towards overt diabetic nepropathy.[1-4] Overt diabetic nephropathy is found in one third of patients with type one diabetes mellitus (DM) and in approximately one fifth from

those with type two DM.[5-10] These patients experience a gradual decrease of kidney function and have a high risk of progression towards end-stage renal disease (ESRD). Currently, DM is the main cause of ESRD, with 45% of the patients included in dialysis programs having this condition.[11-13]

In these circumstances, prevention as well as early diagnosis and treatment of microalbuminuria represent a necessity and justify the introduction of microalbuminuria screening as a standard recommendation in the guidelines for management of DM patients.[14]

However, frequent testing for microalbuminuria in a large diabetes population requires reliable, inexpensive, and fast detection methods. According to its definition, the range of microalbuminuria is usually below the detection threshold of conventional urine strips used for proteinuria. That is why semiquantitative tests were developed for use as screening tools. The effectivenes of these methods is evaluated by determining their sensitivity (the proportion of positive results identified from a group of true-positive cases) and their specificity (the proportion of negative results yielded from a group of real-negative cases).[15-17]

OBJECTIVES

The present study aims to assess the effectiveness of semiquantitative Micral assay (Roche Diagnostics) in detecting microlbuminuria in children and young with type one DM, as compared to quantitative methods for urinary albumin.

PATIENTS AND METHODS

Patients

The study group consisted of 310 type one DM patients, aged between 10 and 30 years, with a DM duration between two and 16 years, admitted in the Cristian Serban Clinical Medical Center for Evaluation and Rehabilitation from Buzias. The inclusion criteria were those recommended for the screening of diabetes nepropathy by leading clinical guidelines.[18-20]

Principle of Micral assay

Micral assay is a specific immunohistochemical assay aimed at measuring semiquantitatively the albumin excretion rate (albuminuria). The albumin from the urine sample binds to the conjugate made up of antibodies against human albumin and an enzyme, beta-galactosidase. The resulting complex is separated and the enzyme reacts with a substrate, generating a red color. The reactant area of the strip is introduced in the urine for 5 seconds and then placed horizontally for 5 minutes. The intensity of the color appearing on the strip is directly proportional to the concentration of urinary albumin. The color of the strip is compared with a referrence color scale from the strip pack. The color scale consists of five color tones corresponding to albumin concentrations of 0, 10, 20, 50, and 100 mg/l. According to producer specifications, albumin concentrations equal to or greater than 20 mg/l is considered positive for microalbuminuria and corresponds to a albumin excretion rate greater than 30 mg/24 hours.

In order to avoid the interpersonal variations and the errors that may appear while interpreting the results, all visual evaluations of the strips were performed by the same person, previously instructed to use and read the assay.

Micral testing was assessed for accuracy with the use of the quantitative immunoturbidimetric method (ITM), considered, together with ELISA and RIA, as a reference method for measuring urinary albumine excretion. Albuminuria was measured using a Cobas Mira analyzer and Tina Quant reactant. Values between 30 and 300 mg/24 hours were considered diagnostic for microalbuminuria.

Urine collection

In 205 patients, urinary albumin was measured with Micral testing using 24 h urine, while in 105 cases it was done using first-void morning urine. In all 310 patients, urinary albumin was measured using ITM , in 24 h urine specimens.

The study did not include patients with a previous diagnosis of overt diabetic nephropathy or chronic renal failure, nor subjects who collected less than 500 ml urine per day or who presented urinary tract infection, marked short-term hyperglycemia or had performed strenuous physical activity prior to testing.[21]

Analysis of results

The results of Micral testing were compared to those obtained with ITM and further classified as true-positive (TP), false-positive (FP), true-negative (TN) or false-negative (FN). Subsequently, we computed the sensitivity, the specificity as well as the positive and negative predictive values (PPV and NPV, respectively) of Micral testing, according to the equations below:

$$\text{Sensitivity} = \frac{TP}{TP + FN} \text{X}\,100$$ [15, 16]

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \text{X}\,100$$ [15, 16]

$$\text{Positive predictive value (PPV)} = \frac{TP}{TP + FP} X\,100$$ [18, 22]

$$\text{Negative predictive value (NPV)} = \frac{TN}{TP + FN} X\,100$$ [18, 23]

RESULTS

In the study group, ITM detected 61 cases (19.6%) that presented microalbuminuria. Micral testing detected 38 out of the 40 samples with microalbuminuria in the 24-h urine group and 19 out of the 21 samples with microalbuminuria in the first-void morning urine group. (Tables 1, 2)

Based on results in the Tables 1 and 2, we have computed sensitivity, specificity, PPV and NPV for Micral corresponding to use of 24 h urine and first-void morning urine samples. (Table 3)

Table 1. Results of Micral assay in the 205 samples of 24-h urine.

ITM		Micral	
		Negative	**Positive**
Positive (Pathologic)	40	False Negative 2	True Positive 38
Negative (Normal)	165	True Negative 138	False Positive 27

Table 2. Results of Micral assay in the 105 samples of first-void morning urine.

ITM		Micral	
		Negative	**Positive**
Positive (Pathologic)	21	False Negative 2	True Positive 19
Negative (Normal)	84	True Negative 76	False Positive 8

Table 3. Parameters of the efficacy of Micral testing.

	24 h urine samples	**First-void urine samples**
Sensitivity (%)	94.6	90.9
Specificity (%)	82.2	90.6
PPV (%)	57.9	71.4
NPV (%)	98.8	97.4

DISCUSSIONS

The sensitivity of Micral testing, a parameter that express the proportion of patients with microalbuminuria detected by this method, was good both for 24 h and first-void morning urine samples (94.6% and 90.9% respectively). On the other hand, specificity, a parameter that describes the proportion of negative cases detected by Micral, was not so high, especially when 24 h urine samples were used (82.2%). Sensibility and specificity values obtained in our study are similar to those published by other papers that span quite large intervals. (Table 4)

Micral specificity is lower as a consequence of a relatively high proportion of false-positive cases. The influence of false-positive results on the efficacy of Micral testing is noticed especially when PPV is computed, a parameter that expresses the probability of true microalbuminuria when a positive result is obtained. PPV was 57.9% for 24 hour urine samples, compared to 71.4% when first-void morning urine was used. As for NPV, a parameter that expresses the probability of the absence of microalbuminuria when the test result is negative, its values were higher for both 24 h and first-void morning urine samples (98.8% and 97.4%, respectively). PPV and NPV values obtained are similar to other published results. (Table 5)

Although PPV of Micral is relatively low, and the proportion of false positive results is 13.1 and 7.6% (for 24 h and first-void morning urine samples, respectively), we should take into consideration the fact that this is a semiquantitative procedure conceived for the screening of microalbuminuria. This means that a positive result obtained with Micral needs to be confirmed using a reference quantitative method that uses urine specimens collected over a certain time period (ELISA, RIA, ITM). Incipient diabetic nephropathy is diagnosed when at least two out of three measurements for microalbuminuria are positive, even when quantitative methods of reference are exclusively used, as albumin excretion rate may vary.[35,36]

On the other hand, NPV reaches very good values (98.8% and 97.4%) as a consequence of a low proportion of false-negative cases (1.9% and 1%, for each type of specimen), that means a patient with negative Micral testing has a high probability for the absence of microalbuminuria. Even in such circumstances, repetead microalbuminuria screening at regular intervals will allow the detection of false negative cases in the preclinical stage. Considering that the evolution from microalbuminuria to overt proteinuria needs on average 3 to 4 years, with large individual variations, it is recommended that the

Table 4. Sensitivity and specificity of Micral testing in studies that assessed the efficacy of the method.

Study	No. of patients	Sensitivity (%)	Specificity (%)
Micral package insert specifications	-	90-99	70-90
Bangstad HJ, Try K, Dahl-Jorgensen K, et al.[24]	186	93.2	82.1
Spooren PF, Lekkerkerker JF, et al.[25]	132	82.0	86.2
Jury DR, Mikkelsen DJ, Glen D, et al.[26]	184	91	97
Gilbert RE, Akdeniz A, Jerums G[27]	298	92.2	92.3
de Grauw WJ, van de Lisdonk EH, et al.[28]	317	67	93
Gosain VV, Gunaga KP, et al.[29]	67	69.5	87.7
Webb DJ, Newman DJ et al.[30]	530	70	87
Mogensen CE, Viberti et al.[31]	meta-analysis	96.7	95.0
Fernandez I, Paez Pinto J, et al.[32]	208	86	88
Scheid DC, McCharty LH, Lawler FH, et al.[33]	meta-analysis	92.3	83.2

Table 5. PPV and NPV of Micral test in efficacy studies.

Study	No. patients	PPV	NPV
Gilbert RE, Akdeniz A, Jerums G [24]	258	86.4	97
Mogensen CE, Viberti, et al. [28]	metaanalysis	78	95
Pegoraro A, Singh A [34]	221	21	99
Leong S O , K F Lui, W Y Ng, Thai A C [35]	100	66.7	94.4

screening of microalbuminuria in those with negative Micral testing should be performed on a yearly basis.[1,37-39] The overall analysis of study results demonstrates that the use of first-void morning urine, besides the less difficult collection compared to 24 h urine, offers the benefit of a more effective Micral testing.

Last but not least, we should also consider the cost of Micral testing in the screening for microalbuminuria, as the price of a test strip is around 2.5 euros. Although it may seem expensive at the first glance, one must be aware that the alternative, represented by the quantitative measurement of microalbuminuria from a timed urine specimen, although more precise, needs a specialized laboratory, equipped with an analyzor suited for albumin assay that requires an expensive reagent. Furthermore, measurement of albumin/ creatinin ratio from the first-morning urine increases costs as urinary creatinine is a supplementary assay. Another advantage of Micral, compared to quantitative albuminuria assays is the fact that the former allows immediate communication of the result without the need for a supplemental visit of the patient.

Furthermore, when cost-benefit ratio is analyzed, one should consider the type of investigated population. If the prevalence of microalbuminuria is increased in the screened population, the costs will be high as positive Micral results need to be confirmed by another assay. On the contrary, the use of Micral for microalbuminuria screening in young type one diabetes patients, who have a low prevalence of diabetic nephropathy, is justified as it is highly effective in excluding negative cases.

CONCLUSIONS

The presented data lead to the conclusion that semiquantitative Micral testing is useful for the screening of microalbuminuria, especially for the exclusion of negative cases but it lacks precision in detecting positive subjects, who require confirmation using quantitative methods.[40] The inclusion of Micral testing in an algorythm for early detection of microalbuminuria allows the diagnosis of patients with incipient diabetic nephropathy. However, this does not necesarily means a better outcome for these patients, unless prompt therapeutic measures are undertaken by the attending physicians. In this regard, it is necessary to emphasize the education of treating physicians to enable them to start early therapy, based on microalbuminuria results, with the aim to prevent the evolution of diabetic nephropathy towards ESRD.

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