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ORIGINAL ARTICLE

# Detection of microalbuminuria in non-insulin dependent diabetes mellitus (NIDDM) patients without overt proteinuria by a semiquantitative albumin-creatinine urine strips

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## ABSTRACT

*Microalbuminuria is the hallmark of the reversible stage of incipient diabetic nephropathy. A cost-effective and convenient bedside screening test is essential to detect this phase. We used Clinitek 50® which is a semiquantitative strip test to check spot urine sample from 81 patients with albustix one plus or less. The incidence of Clinitek 50® microalbuminuria was 17%, 18.2% and 75% in 47, 22 and 12 patients with albustix negative, trace or one plus respectively. Nineteen and 13 of the 21 Clinitek 50® positive patients were checked for spot urine DCA 2000® and two 12-hour urine collection for immunoassay respectively. Around 60% of these samples fell into the microalbuminuria range and 40% into the overt albuminuria range by either technique. There was no false positive of Clinitek 50®. The lowest range of microalbuminuria detected by Clinitek 50® was 27 μg/minute (38 mg/day). We concluded that Clinitek 50® is a useful screening test as it is nonexpensive, easily operated and has a sensitivity close to the lower range of microalbuminuria.*

*Key words: Microalbuminuria, Non-insulin dependent diabetes mellitus ( NIDDM)*

## 中文摘要

微量白蛋白尿是糖尿病腎病患者初期可逆轉階段的重要標誌，對此階段經濟方便的床頭篩選試驗十分重要。我們利用 Clinitek50® 對 81 名尿蛋白定性一個加號(+)或稍少的患者的尿樣本進行了半定量條帶試驗。結果顯示，在 47 名、22 名及 12 名尿蛋白常規顯示為陰性、微量及一個加號的患者中，其 Clinitek50® 微量白尿白率分別為 17%、18.2% 及 75%。在 21 位 Clinitek50® 陽性患者中，19 位及 13 位分別接受了點尿樣 DCA2000® 及兩次 12 小時尿量收集進行免疫分析。兩種檢測技術表明大約 60% 的樣本在微量白蛋白尿範圍，40% 在臨床白蛋白尿範圍。Clinitek50® 法無假陽性率。Clinitek50® 法檢測微量白蛋白尿的最低限度為 27μg/ 分(38mg/ 天)。因此，我們認為 Clinitek50® 是一種既廉宜又方便，敏感度與微量白蛋白尿最低量相近的一種有用的臨床篩選試驗。

## INTRODUCTION

Microalbuminuria defined as urinary albumin excretion rate of 30 to 300 mg/day (or 20-200 μg/minute) is one of the most important single predictor for future development of overt diabetic nephropathy (1,2,3). The microalbuminuric phase is a reversible stage in the development of diabetic nephropathy when appropriate intervention is being instituted and is regarded as incipient diabetic nephropathy (4-6). Consequently, early detection and prompt treatment of incipient diabetic nephropathy is of ultimate importance in managing diabetic patients (7-13). However, detection of microalbuminuria requires the expensive immunoassay

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(IA) measurement of 24-hour urine sample which is known to be liable to inaccurate collection (14,15).

In the past decade, albumin-creatinine ratio (ACR) becomes an important tool to quantify microalbuminuria and to avoid inaccuracies of 24-hour urine collection (16-19). Clinitek 50® (Bayer, Elkhart, USA) is one of the current semiquantitative screening tool for microalbuminuria commercially available. This study is to compare Clinitek 50® with other quantitative methods in detecting microalbuminuria and to study the prevalence of incipient diabetic nephropathy in noninsulin dependent diabetes mellitus (NIDDM) patients without overt proteinuria.

## METHODS

All patients with NIDDM attending the general medical clinic at Tung Wah Hospital from 30th October 1998 to 27th November 1998 were screened for their previous albustix results. Patients with previous albustix results of one plus or less (equivalent to proteinuria ≤ 0.3 g/L) were recruited in this study. During their routine follow-up, a spot urine albustix was examined by an experienced nurse. Urine from patients with the spot urine albustix result one plus or less would be checked with Clinitek 50®. Urine with ACR between 3.4 mg/mmol to 33.9 mg/mmol by Clinitek 50® was immediately checked with DCA 2000® (Bayer, Elkhart, USA). Patients were also instructed to collect two overnight 12-hour urine samples for assay of microalbuminuria with IA in Clinical Biochemistry Laboratory at Queen Mary Hospital.

Clinitek 50® is a semiquantitative test using plastic strips with two reagent-impregnate pads, one to measure albumin and another creatinine. An ACR in mg albumin per mmol creatinine is determined. The color range of the albumin test is based on the binding of albumin with a sulfonephthalein dye, resulting in a color shift at a constant pH. This is referred to as the "Protein Error" of pH indicators. The creatinine test is based on an enzyme-linked activity of a copper creatinine complex. The copper creatinine complex reacts with a hydroperoxide resulted in a color change ranging from deep gold (30 mg/dL) to deep green (300 mg/dL). Clinitek 50® provides a categorized result of less than 3.4, 3.4 to 33.9 and more than 33.9 mg albumin/mmol creatinine. ACR of 3.4 to 33.9 is regarded as the microalbuminuria range.

DCA 2000® is a quantitative test employing an immunoturbidimetric technique to measure the ACR. A purified polyclonal goat anti-human albumin anti-serum antibody binds with albumin in the presence of polyethylene glycol. The complex formed causes turbidity which is measured as absorbance. The creatinine is assayed by colorimetric method based on Benedict/Behre reaction. Creatinine forms a colored complex with 3,5-dinitrobenzoate at alkaline pH by addition of potassium hydroxide. Absorbance of this colored complex is then measured at 531 nm. ACR of 3.4-33.9 mg/mmol is regarded as the microalbuminuria range. Both Clinitek 50® and DCA 2000® tests was conducted by the same doctor in the out-patient department without knowing the identity of the patients.

Two timed overnight 12-hour urine samples were collected instead of traditional 24-hour urine for IA of albuminuria. It replaced the standard 24-hour collection in order to reduce the error from non-compliant collection. It also fulfills the requirement for the proper diagnosis of incipient diabetic nephropathy that requires at least two consecutive urine samples of albumin excretion rate of 20 to 200 μg/minute as day to day variation of albumin excretion varies between 30% to 50% (20). Urinary albumin was quantitated with the Beckman Array 360 Analyzer (Beckman-Coulter Inc., USA) employing the rate nephelometry method. The sensitivity of the method is 2 mg/L.

## RESULTS

Urine samples were collected from 81 patients who fulfilled the inclusion criteria. Of the 81 specimens, albustix was negative in 47 samples, trace in 22 and one plus in 12 samples. The incidence of microalbuminuria by Clinitek 50® method was 17%, 18.2% and 75% (which also included two cases of Clinitek 50® > 33.9 mg/mmol) in the three groups of patients with negative, trace and one plus albustix test respectively (Table 1). None of the urine sample tested negative or trace by albustix has ACR more than 33.9 mg/mmol by Clinitek 50®.

Of the 21 samples with Clinitek 50® more than 3.4 mg/mmol, 19 were in the microalbuminuria range (3.4 mmol/L - 33.9 mmol/L). Eighteen samples were tested with DCA 2000®. Ten samples (55.6%) fell into the DCA 2000® microalbuminuria and eight samples (44.4%) fell into the macroalbuminuria range (> 33.9 mg/mmol). Only 13 patients had sent their two 12-hour urine collections, of these eight (61.5%) were microalbuminuric and five (38.5%) had exceeded the microalbuminuria range, and all of the two consecutive samples of the same patient showed the same range of microalbuminuria or macroalbuminuria. None of the Clinitek 50® positive patients was negative for either DCA 2000® or IA. The range of microalbuminuria detected by Clinitek 50® was between 27 μg/minute to 2250 μg/minute (38.8 mg/day-3.24 g/day, mean ±SD 603 ±871 mg/day and median 253 mg/day) according to IA,

**Table 1.** Result of Albustix, Clinitek 50®, DCA 2000® of spot urine and immunoassay of timed urine collection in 81 NIDDM patients.

| Albustix n = 81 | Clinitek 50® n = 81 | DCA 2000® n = 19 | Timed urine collection n = 13 |
|---|---|---|---|
| (0) | (0) n = 39 | NA | NA |
| n = 47 | (+) n = 8 | (+) n = 4 | (+) n = 4 |
| | | (++) n = 4 | (++) n = 2 |
| | | | Missing n = 2 |
| (Trace) | (0) n = 18 | NA | NA |
| n = 22 | (+) n = 4 | (+) n = 3 | (+) n = 2 |
| | | (++) n = 1 | (++) n = 1 |
| | | | Missing n = 1 |
| (+) | (0) n = 3 | NA | NA |
| n = 12 | (+) n = 7 | (+) n = 3 | (+) n = 2 |
| | | (++) n = 3 | (++) n = 2 |
| | | Missing n = 1 | Missing n = 3 |
| | (++) n = 2 | (+) n = 1 | |
| | | Missing n = 1 | Missing n = 2 |

Abbre: NA = Not applicable
Clinitek 50® and DCA 2000®: (0) = ACR < 3.4 mg/mmol,
(+) = ACR 3.4-33.9 mg/mmol (++) = ACR > 33.9 mg/mmol
Timed urine collection :
(+) = Albumin excretion rate 20-200 µg/minute
(++) = Albumin excretion rate > 200 µg/minute

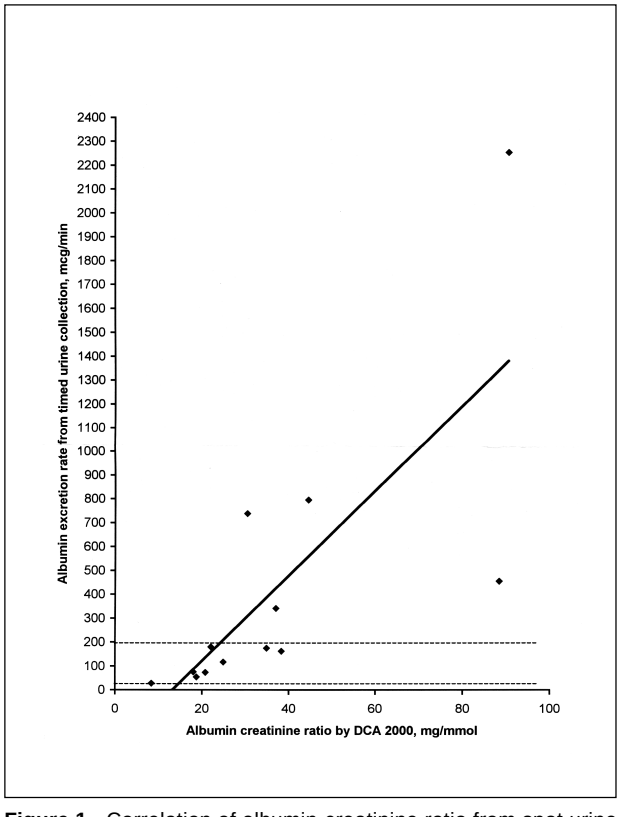


**Figure 1.** Correlation of albumin-creatinine ratio from spot urine by DCA 2000® and albumin excretion rate from two 12-hour urine collections by immunoassay. The solid line represents the line of correlation with correlation coefficient, r = 0.747 ($p$ = 0.003). The area between the two dotted line represents the microalbuminuric range of albumin excretion rate.

**Table 2.** Comparison of demographic and clinical data of 81 NIDDM patients with normal and abnormal (ACR >3.4 mg/mmol) Clinitek 50® results.

| | Normal | Abnormal | *P* value |
|---|---|---|---|
| No. of patients | 60 | 21 | |
| Age | 61.3 ±11.7 | 68.6 ±9.5 | 0.012* |
| Sex, M:F | 30:30 | 13:8 | NS |
| Years with NIDDM | 6.7 ±6.1 | 7.0 ±4.4 | NS |
| Fasting blood sugar, mmol/L | 8.5 ±3.2 | 7.7 ±2.7 | NS |
| $HbA_1C$, % | 8.1 ±1.7 | 7.8 ±1.8 | NS |
| Mean BP, mmHg | 96.8 ±12.5 | 94.3 ±12.4 | NS |

*by two sample t-test
Ns = Not significant

and the ACR range was between 8.36 mg/mmol to 90.7 mg/mmol according to DCA 2000®. There was no false positive detected by Clinitek 50® and there was a significant correlation between the DCA 2000® and IA results (r = 0.747, $p$ = 0.003) (Fig. 1).

The demographic and clinical data of patients with positive and negative Clinitek 50® were shown in table 2. With logistic regression, age of patient was the only significant risk factor for the occurrence of microalbuminuria or overt proteinuria in these 81 patients (odds ratio = 1.065, $p$ = 0.031).

## DICUSSIONS

Proteinuria can be measured in random samples or 24-hour collections. Inaccurate urine collection is probably the most common source of error in quantifying protein excretion in timed collections (7-13). Total urinary protein concentration can be estimated with strips at bedside. There are dipsticks with sensitivity to urinary protein concentrations as low as 100 mg/L to 200 mg/L (21). However, at these low levels, the major constituent of urinary protein is usually Tamm-Horsfall protein and may not indicate the presence of albumin. On the other hand, when urine volume is high and the urine is maximally diluted, a relatively large amount of protein may be undetected and false negative result will arise. The consistency of results with the same sample assessed repeatedly and the precision of reagent strip tests of urinary total protein concentration are generally poor (21,22). Inconsistency was dependent on the experience of the operator. Inconsistency among experienced and inexperienced technologists can be up to 33% and 93% respectively (22). ACR has been used to correct problems arising out of variability in urine volume and concentration. There is a high degree of correlation between 24-hour urinary albumin excretion and ACR in random, single-voided urine samples in patients with renal disease (23,24). Although ACR may be more

quantitative than a simple dipstick test, their use has a number of limitations. For example, ACR taken in the morning, first-void urine samples may underestimate 24-hour protein excretion because the reduction in proteinuria usually occurs at night (25). The measurement of creatinine level may introduce another source of error. The errors of two measurements combined will be greater than the error of either one alone (25). Despite these limitations, the ACR may be useful especially in individuals in whom urine collection is difficult.

Microalbuminuria is the first stage of diabetic nephropathy that can be detectable clinically. This stage of incipient diabetic nephropathy is still potentially salvageable (4-6). More than 30% of insulin dependent diabetes mellitus (IDDM) patients (7,26-28) and 25% of NIDDM patients will develop overt diabetic nephropathy ultimately (8,29-31). The routine use of albustix at clinic visits is convenient and inexpensive but is unfortunately insensitive for detecting microalbuminuria. The traditional 24-hour urine collection for microalbumin assay is too time consuming, subject to timing and collection inaccuracies (7-13), and is also too expensive for screening large number of patients with negative albustix. Therefore it is not a suitable screening test. A cost-effective test which allows large scale screening in an out-patient basis is of vital importance to detect this potentially salvageable phase of incipient diabetic nephropathy.

In our small scale survey, we find that there is a good concordance between the Clinitek 50® results with either DCA 2000® or IA from two 12-hour urine collections. Although the specificity and sensitivity of this test cannot be generated from our data, the range it covered was as low as 38 mg/day. Therefore we believe that Clinitek 50® is useful for detection of microalbuminuria. The test is easy and can be performed at bedside. It takes 20 seconds per test and costs only HK$15 per strip. The DCA 2000® is much more time-consuming. It takes around 10 minutes for one assay and costs HK$73 per test.

The prevalence of microalbuminuria as detected by Clinitek 50® in this study was around 18% for both groups of patients with negative or trace albustix test. The distinction between negative and trace albustix test is largely subjective and difficult. They may probably be better considered under the same category. Around 40% of these Clinitek 50® microalbuminuric patients actually had proteinuria exceeding the microalbuminuria range by DCR 2000® or by IA. This may mean that around 11% and 7% of patients with negative or trace albustix results have already developed incipient and overt diabetic nephropathy respectively. This prevalence of microalbuminuria in patients without overt proteinuria is quite similar to those reported by Tiu et al in Hong Kong (14.6%-16%) (32) and by Leong et al in Singapore (18%) (33). Although Clinitek 50® does not distinguish microalbuminuria from overt proteinuria very well, it is still useful in detecting low grade microalbuminuria as it was positive in a patient with albumin excretion rate as low as 27 μg/minute (38 mg/day) in this study. We concluded that Clinitek 50® is a convenient and cost-effective tool for screening of microalbuminuria in patients with negative or trace albustix results at regular intervals. If it is Clintek 50® positive, the result should further be confirmed by two 24-hour or 12-hour overnight urine collections for microalbuminuria assay or other more specific quantitative assays of spot urine sample like DCA 2000®.

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