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NEW MARKER FOR THE EARLY DIAGNOSIS OF NEPHROPATHY ASSOCIATED WITH TYPE II DIABETES MELLITUS

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ABSTRACT:

In this study the relationship between values of serum cystatin C or serum creatinine and prognostic stages of type II diabetic nephropathy was determined. Blood samples from 87 patients with type II diabetes were obtained from Nephrology Department, Zagazig University Hospitals, Zagazig, Egypt.

Patients were categorized into five stages as follows: stage I, stage II, stage III_a, stage III_b and stage IV according to the concentration of albumin in their 24 hours urines.

The values of both serum cystatin C and creatinine were measured. It was found that, the mean levels of serum cystatin C in stage III_a were significantly higher than those in stages I or II ($p < 0.00001$/ $p < 0.0005$ respectively).

The mean values of serum cystatin C in stages III_b and IV were also significantly higher than those in stage I ($p < 0.00001$).

However, the mean values of serum creatinine in stage III_a were not significantly higher than those in stages I or II. The values of serum creatinine in stages III_b and IV were significantly higher than those in stage I ($p < 0.00001$).

The Receiver Operating Characteristic (ROC) plot indicated that, serum cystatin C may be superior to serum creatinine for detecting impaired glomerular filtration rate (GFR).

It appears that the values of serum cystatin C may be of value in predicting prognostic stages of patients with type II diabetic nephroparhy.

INTRODUCTION

Diabetic nephropathy is one of the most serious complications in type II diabetes mellitus. In clinical practice, progressive kidney failure often goes unrecognized until a patient has lost >50% of normal kidney function. This is in part because of the lack of an easy method to measure the glomerular filtration rate (GFR), which is the best overall index of renal function in health and disease[1].

Many techniques have been used to detect early renal impairment in diabetes mellitus and other diseases.

Microalbuminuria is commonly seen in patients with type II diabetes mellitus. The albumin excretion rate per 24 hours (AER) is a useful indicator, but a day- to - day variation of up to 40% limits its use for that purpose[2].

GFR using exogenous substances such as inulin, ^{51}Cr-EDTA and ^{125}I-iothalamate are considered to be the gold standard, but are rarely used clinically because of the cost and the cumbersome procedures involved[3].

Creatinine clearance calculated with the Cockcroft and Gault formula[4] is used also for the measurement of GFR.

C and G formula = [140 – age (years)] body weight (kg) / [0.815 x plasma creatinine (μ mol/L)] = creatinine clearance ml / min. / 1.73 m2.

The formula is also inaccurate in patients with liver diseases, muscle wasting, oedema or extreme adiposity[5].

Serum creatinine is widely used for the rapid assessment of GFR, however GFR may be inadequately estimated due to differences in sex, age, muscle mass and tubular secretion of creatinine[6].

Recently, human cystatin C, which is a nonglycosylated, low molecular weight, basic protein that belongs to the superfamily of cysteine proteinase inhibitors. It is steadily expressed in all nucleated cells, and is present at relatively high concentration in body fluids, including serum, urine, seminal fluid, cerebrospinal fluid and synovial fluid[7].

Cystatin C is produced at a constant rate[8], and is freely filtered by the glomerulus because of its small size and positive charge. Unlike creatinine, cystatin C is not secreted by renal tubular epithelial cells[9]. Cystatin C values do not appear to be affected by muscle mass[10]. The characteristics of cystatin C may consider it an endogenous marker for GFR assessment.

This study was designed to test whether serum cystatin C can replace serum creatinine for the early assessment of diabetic nehropathy.

SUBJECTS AND METHODS

Patients:

Blood samples from 87 patients (Their ages ranged from 35-55 years) with type II diabetes mellitus were obtained from Nephrology Department, Zagazig University Hospitals, Zagazig, Egypt. and duration of diabetes from 18-23 years.

Patients were classified into five stages according to the concentration of albumin in their 24 hours urines as follows:

Stage **I** (34 patients) normoalbuminuria, used as control group

Stage **II** (15 patients) microalbuminuria

Stage **III_a** (16 patients) macroalbuminuria

Stage **III_b** (8 patients) were macroalbuminuria with renal dysfunction.

Stage **IV** (14 patients) have renal failure.

Methods:

Determination of serum cystatin C and serum creatinine:

1- Seum cystatin C was determined by ELISA technique[11].

2- Serum creatinine was measured according to the method of Jaffe[12].

Statistical Analysis:

Data are presented as mean ± S.D.

The significant difference between groups was evaluated by student's "t" test at $P < 0.0005$ and $P < 0.00001$ respectively.

RESULTS

Comparison between mean values of cystatin C and creatinine in sera of patients with type II diabetic nephropathy:

The mean values of serum cystatin C and serum creatinine in patients with type II diabetes mellitus are shown in tables (1 and 2).

The mean values of serum cystatin C in group III_a was significantly higher than those in group I or II ($P < 0.00001$, $P < 0.0005$, respectively).

The mean values of serum cystatin C in group III_b and group IV were also significantly higher than those in group I ($P < 0.00001$).

There were no significant changes in the values of serum cystatin C between group I and II (Fig. 1).

However, the mean values of serum creatinine in group III_a were not significantly higher than those in group I or II.

The values of serum creatinine in group III_b and group IV were significantly higher than those in group I ($P < 0.00001$).

There were no significant changes in the values of serum creatinine between groups I and II (Fig.2).

ROC curve analysis:

Receiver Operating characteristic (ROC) curve analysis was performed to determine if serum cystatin C is useful in predicting the earlier prognostic stage (group II) of type II diabetic nephropathy.

ROC plots demonstrated that the area under the curve (AUC) of cystatin C (0.76) was greater than that of creatinine (0.66) (Fig. 3).

To distinguish between groups II and III_a sensitivity and specificity of serum cystatin C were better than those of serum creatinine.

Table 1: Mean values of serum cystatin C in patients with Type II diabetic nephropathy.

Stage	Number of patients	Serum cystatin C Mean ± S.D (mg/L)
I	34	0.679 ± 0.096
II	15	0.705 ± 0.118
III_a	16	0.896 ± 0.242**
III_b	8	1.506 ± 0.339**
IV	14	3.049 ± 0.961**

* Significant difference from control at $P < 0.0005$.
** Significant difference from control at $P < 0.00001$.

Table 2: Mean values of serum creatinine in patients with type II diabetic nephropathy

Stage	Number of patients	Serum creatinine Mean ± S.D (mg/dl.)
I	34	0.723 ± 0.131
II	15	0.722 ± 0.133
III_a	16	0.823 ± 0.226
III_b	8	1.562 ± 0.240**
IV	14	4.250 ± 2.459**

* Significant difference from control at $P < 0.0005$.
** Significant difference from control at $P < 0.00001$.

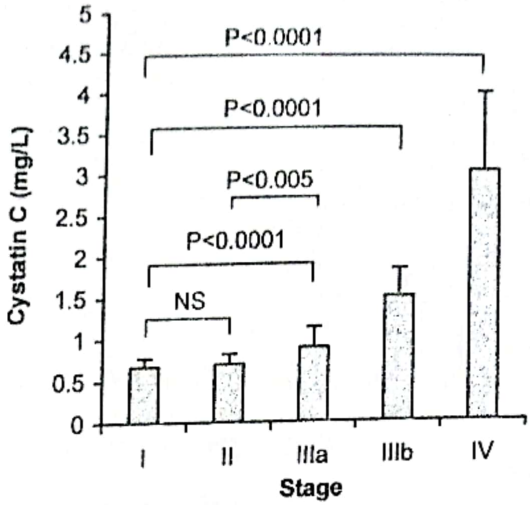

Fig. 1: Serum values of cystatin C in patients in different stages of type II diabetic nephropathy.

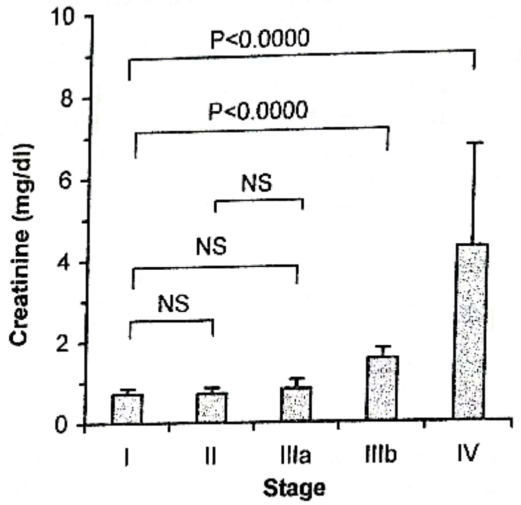

Fig. 2: Relationship between values of serum creatinine in patients in different stages of type II diabetic nephropathy.

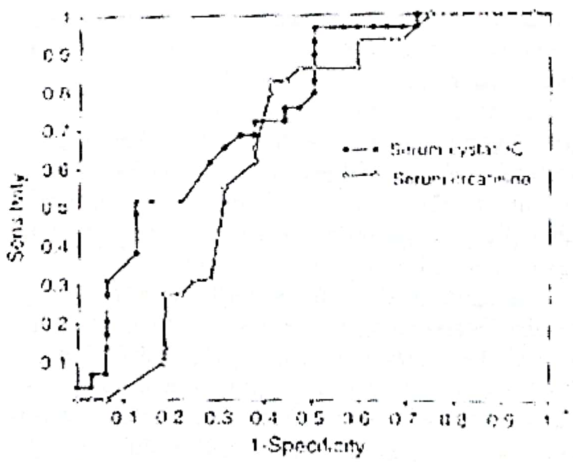

Fig. 3: Receiver Operating Characteristic (ROC) plots to assess the diagnostic efficiency of serum cystatin C and serum creatinine in distinguishing between stages II and III_a.

DISCUSSION

Evaluation of GFR in patients with diabetes mellitus is important because a significant proportion of patients manifest glomerular hyperfiltration in the first few years of the disease, but as diabetes progresses and microalbuminuria occurs the GFR begins to decline[13].

In recent years there have been several reports suggesting that serum cystatin C measurement correlates with the GFR. The authors reported a relationship between values of serum cystatin C and prognostic stages in patients with diabetic nephropathy[14].

They reported also that, the values of serum cystatin C were statistically more correlated with the prognostic stages of diabetic nephropathy than those of serum creatinine[15].

In our study the mean values of serum cystatin C in stage III_a patients were significantly higher than those in stage I. The mean values of serum cystatin C in patients in stages III_b and IV were also significantly higher than those in stage I (table 1).

However the mean values of serum creatinine in stage III_a patients were not significantly higher than those in stage I. The levels of serum creatinine in stages III_b and IV were significantly higher than those in stage I (Table 2).

The diagnostic utility of Cys C is becoming better appreciated, although its superiority to sCr measurement in all patient populations has not yet been clearly established. Several investigations have suggested that in patients with various renal diseases, Cys C is at least as useful as sCr determination in detecting declining GFR[16].

Kazama et al.[17] determined the GFR in 212 patients with a variety of renal diseases. They compared this measured GFR with the CCr, sCr and sCys C. it was concluded that sCys C is superior to CCr when subclinical renal dysfunction is present. Likewise Nitta et al.[18] studied GFR in 140 patients with various renal diseases, he also found that, sCys C measurement identifies patients with mild reductions in GFR more accurately compared to sCr determination.

Recent investigations support the contention that, Cys C serves as reliably as sCr as a marker of renal function in elderly and pediatric populations. In both populations, low muscle mass may produce low sCr values that do not reflect true underlying GFR, and may obscure small changes in true GFR[16].

Burkhardt et al.,[19] measured GFR in an elderly population and compared results with CCr, CGF and sCys C. he determined that, sCys C and CGF were slightly more adequate than CCr. It appears that, at least in elderly, sCys C offers an equivalent but not necessarily superior measure of GFR. Reduced muscle mass is also present in those with major motor spinal cord injuries, just as it may be in the elderly. Thomassen et al.[20] evaluate the clinical usefulness of sCys C determination in a group of men and women with major spinal cord injuries, as measured against sCr and determination of GFR, he found that in this population sCys C was a more reliable marker of renal function than was sCr.

Shimuza et al.,[21] reported that, sCys C estimation may be clinically useful for identifying and monitoring mild or early renal dysfunction in certain patients.

Mussap et al.,[22] demonstrated that when compared with sCr, the reciprocal of sCys C correlated more strongly with GFR than did either the reciprocal of sCr and CGF. This allowed a distinction to be made between type II diabetics with normal or slightly reduced GFR.

In addition to the information that sCys C gives on the presence or absence of even mild renal dysfunction, it does this irrespective of sex, age, weight, height and muscle mass. This makes sCys C more specific than serum creatinine in evaluating renal function[23, 24].

Receiver Operating Characteristic (ROC) curve analysis demonstrated that, the area under the curve of serum cystatin C (0.76) was greater than that of serum creatinine (0.66). Thus sensitivity and specificity of serum cystatin C were better than those of serum creatinine. It appears that the levels of serum cystatin C can predict the early prognostic stages of patients with type II diabetic nephropathy, but further studies are required to confirm its usefulness as a screening test capable of use as an early indicator and predictor of the development of diabetic nephropathy.

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دلالة جديدة للتشخيص المبكر لأمراض الجهاز البولى المصاحبة لمرض السكر من النوع الثانى

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تـم فى هذه الدراسة إلقاء الضوء على العلاقة بين مستوى كل من السيستاتين ج أو الكرياتنين فى مصل الدم وبدايات ظهور اضطرابات الجهاز البولى لدى مرضى السكرى من النوع الثانى.

تـم الحصـول علـى عينات الدم من ٨٧ مريض مصابون بمرض السكر من النوع الثانى من قسم المسالك البولية – مستشـفيات جامعـة الزقازيق – الزقازيق – مصر حيث يعالج هؤلاء المرضى بأدوية لخفض السكر عن طريق الفم وتم تقسيمهم إلى ٥ مجموعات (حسب تركيز الزلال فى عينة بول ٢٤ ساعة الخاصة بكل مريض منهم) وهى: مجموعة I ، II ، IIIا ، IIIب و IV . تم قياس مستوى كل من السيستاتين ج وكذلك الكرياتنين فى مصل الدم ، وقد وجدنا الآتى: مستوى السيستاتين ج فى المجموعة IIIا كان ذو دلالة إحصائية أعلى منه فى المجموعات I و II ، كذلك فى المجموعات IIIب و V كان ذو دلالة إحصائية أعلى منه فى المجموعة I.

أما بالنسبة لكرياتنين المصل فقد أظهر البحث أن مستوى مصل الكرياتنين فى المجموعة III أ ليس له دلالة إحصائية عنه فى المجموعات I و II لكنه فى المجموعات III ب ، IV كان ذو دلالة إحصائية عنه فى المجموعة I.

وقـد أثبتت الإحصائيات أن قياس السيستاتين ج فى مصل الدم يعتبر أفضل من قياس الكرياتنين فى مصل الدم وذلك للكشف المبكر عن أولى بوادر اضطرابات الجهاز البولى لدى مرضى البول السكرى من النوع الثانى.