Int J Clin Exp Med 2016;9(10):19919-19924
www.ijcem.com /ISSN:1940-5901/IJCEM0031153

Original Article

Comparison of renal dynamic imaging and two plasma sample method in determining glomerular filtration rate in patients with diabetic nephropathy

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Received April 25, 2016; Accepted May 10, 2016; Epub October 15, 2016; Published October 30, 2016

Abstract: Objective: This study aims to compare the application significance of renal dynamic imaging (RDI) and two plasma sample (TPS) method in determining glomerular filtration rate (GFR) in patients with diabetic nephropathy. Methods: A total of 68 patients who received medical service in the Endocrinology Department of our hospital were enrolled into this study. These patients were divided into three groups: early nephropathy group (DN1 group, *n*=23), clinical nephropathy group (DN2 group, *n*=23) and uremia group (DN3 group, *n*=22). In addition, 22 healthy subjects were enrolled and assigned as control group (DN0 group). GFRs of all subjects were determined by both RDI and TPS method, and the correlation between results and determination methods were analyzed. Results: GFRs (gGFRs and tGFRs) in the DN0, DN1 and DN2 groups were determined by RDI and TPS, and the difference was not statistically significant ($P>0.05$). Differences between gGFR and tGFR in the DN3 group was statistically significant ($P<0.05$). Correlation coefficients between GFRs determined by RDI and TPS in the DN0, DN1, DN2 and DN3 groups were 0.999, 0.947, 0.925 and 0.999, respectively. All values exhibited a significant positive correlation ($P<0.05$). Conclusions: Both RDI and TPS can sensitively reveal GFR changes in early and clinical nephropathy. However, RDI revealed poor accuracy and low sensitivity in the diagnosis of diabetic nephropathy in the uremic phase, and medical history combined with other examination methods should be applied in the diagnosis.

Keywords: Renal dynamic imaging, two plasma sample (TPS) method, glomerular filtration rate, diabetic nephropathy

Introduction

Diabetic nephropathy (DN) is one of the severe complications in type-II diabetes mellitus [1]. At present, the diagnosis still relies on urinary albumin excretion rate (UAER) [2]; and a diagnosis standard that rely on glomerular filtration rate (GFR) has not established. Since UAER is vulnerable to infection, exercise and other diseases, it is urgently necessary to establish a stable diagnosis standard that relies on GFR. Furthermore, the accuracy of the determined GFR is also very important. At present, a variety of methods can be used to determine GFR. The two plasma sample method (TPS) [3] has been recommended by the American College of Nuclear Medicine as the gold standard for determining GFR, due to its simple operation, high accuracy and stability. In recent years, this has become the reference standard for determining GFR. Renal dynamic imaging (RDI) has been widely used in clinical practice due to its capacity to determine renal blood perfusion and total and partial GFR [4]. However, the accuracy of RDI in determining GFR remains to be confirmed [5]. In particular, few studies that determined GFR using RDI in different populations have been reported [6]. In this study, with TPS as a reference, the accuracy of RDI in determining GFR in patients with diabetic nephropathy was evaluated [7]. Details are reported as follows.

Materials and methods

General information

A total of 68 DN patients admitted in the Endocrinology Department of our hospital were enrolled into this study. Among these patients,

38 were male and 30 were female. The age of these patients ranged within 35-78 years old, with an average age of 55.73±10.32 years old. The disease course ranged with 5.5-30.6 years, with an average of 12.81±6.23 years. According to UAER, these patients were divided into three groups: early nephropathy group (DN1 group, *n*=23), clinical nephropathy group (DN2 group, *n*=23), and uremia group (DN3 group, *n*=22). In addition, 22 subjects with normal physical examination results were enrolled as the control group (DN0 group). Among these controls, 12 were male and 10 were female. The age of participants in the control group ranged within 40-75 years, with an average age of 43.58±5.98 years.

Inclusion criteria: The selected patients were included into the study based on the following conditions: (1) patients in line with the 2010 diabetes diagnostic criteria of the American Diabetes Association (ADA); (2) patients in line with the diagnostic criteria of diabetic nephropathy; (3) patients without tumor, cirrhosis and other severe complications; (4) patients who have an understanding of this study and provides a signed informed consent.

Exclusion criteria: (1) patients with recent unstable glycemic control, and are very likely to develop diabetic ketoacidosis or a diabetic nonketotic hyperosmolar state; (2) patients with DN combined with active tuberculosis, chronic hepatitis, urinary tract infection and other kidney diseases; (3) patients who recently recieved renal-damaged drugs, hypotensors or lipid-lowering drugs; (4) patients with DN combined with other acute complications; (5) patients with poor blood sugar control, which have large fluctuations; (6) patients with severe complications and could not continue to complete the examination.

Diagnostic criteria

With reference to the 2010 diabetes diagnostic criteria of ADA: Glycated hemoglobin levels, fasting blood glucose concentration, and the 2nd hour glucose level after oral glucose tolerance test were not less than 6.5%, 7.0 mmol/L and 11.1 mmol/L, respectively. Fasting refers to patients with no calorie intake in no less than eight hours, or patients with typical high blood sugar or acute hyperglycemia symptoms that have random blood glucose level not less than 11.1 mmol/L. In the absence of proven high blood sugar, glycated hemoglobin level, fasting blood glucose concentration and the 2nd hour glucose level after oral glucose tolerance test should be repeatedly performed to confirm whether these indexes are beyond the standard.

With reference diagnostic criteria of DN: (1) Early DN: The diagnosis was mainly based on the increase in UAER (normal <10 μg/min). The diagnosis of DN requires that UAER level ranging between 20 and 200 μg/min should occur twice during consecutive uronoscopy within a six-month period. At the same time, other reasons causing its increase should be ruled out, such as urinary tract infection, exercise, primary hypertension, heart failure and water load increase. (2) Clinical DN: history of diabetes mellitus; UAER >200 μg/minute, and with intermittent or continuous clinical albuminuria (urine protein positive) caused by other causes except for the one previously mentioned, which may be combined with edema, hypertension and renal function impairment. (3) Uremia: most of the nephrons develop atresia, UAER decreases, serum creatinine increases, and blood pressure increases.

Instrument and materials

Philips Bright View SPECT was used as the imaging instrument. Marking diethylenetriamine-pentaacetate (DTPA) was provided by the Beijing Shi Hong Drug Development Center, with a radiochemical purity >95%. The dose of ^{99m}TC-DTPA was 185 MBq, and the volume was less than 1 ml. Automatic dual probe radioimmunity gamma counter.

Methods

GFRs of all subjects were determined by both RDI and TPS method, and urinary microalbumin (mAlb) in all subjects was detected by immunoradiometric assay.

Renal dynamic imaging [8]: All subjects took their breakfast in the usual manner, drunk 300-500 ml of water 30 minutes before imaging, emptied their bladder immediately before imaging, and their heights and weights were recorded. The subjects were laid in the supine position, the probe was placed under the examination couch, and the two kidneys and

Table 1. General data and clinical data in each group

	DN0	DN1	DN2	DN3
Number	22	23	23	22
Gender ratio	12/10	13/10	12/10	12/10
Age (years old)	43.58±5.98	54.28±5.87*	65.43±6.01*	67.24±6.47*
Height (cm)	162.03±2.57	162.38±2.50	161.97±2.51	161.78±2.67
Weight (kg)	67.76±3.51	65.26±3.45*	63.47±3.47*	62.53±4.01*
UAER (ug/min)	12.7±7.1	98.1±73.1*	280.2±59.8*	254.7±89.3*

Note: compared with DN0, *P<0.05.

Table 2. Comparison of gGFR and tGFR in each group

Groups	n	gGFR (l/min)	tGFR (ml/min)	t value	*p* value	r value	*p* value
DN0	22	92.12±11.25	92.09±11.30	0.231	0.820	0.999	0.000
DN1	23	96.57±3.55*	96.35±3.96*	0.817	0.423	0.947	0.000
DN2	23	58.47±4.36*	58.84±4.65*	0.997	0.330	0.925	0.000
DN3	22	30.03±5.54*	25.29±9.03*	4.148	0.000	0.835	0.000

Note: compared with DN0, *P<0.05.

bladder were confirmed on the site in the visual field of the probe. Approximately 1 ml of ^{99m}TC-DTPA (approximately 185 MBq/1 ml) was administered by bolus injection at the elbow vein of the patient [9]. The dynamic data acquisition of emission computed tomography (ECT) was immediately started, which lasted for 20 minutes. Then, radioactive counts of empty and full syringe before and after the acquisition was recorded to calculate the radioactive counts injected [10]. After acquisition, matching software was used to process the images. The total GFR of the two kidneys were calculated using Gate's method, was expressed by a standardized body surface area (1.73 m^2), and recorded as gGFR.

Two plasma sample method [11]: During the renal dynamic scan, the time used to determine the radioactive counts under full or empty syringe was recorded in detail. At the second hour (T1, min; P1, CPM) and fourth hour (T2, min; P2, CPM) after intravenous injection of ^{99m}TC-DTPA [12], 4 ml of blood was obtained from the forearm vein of the other side. Then, blood samples underwent anticoagulation by heparin, and centrifuged at 2,000 r/min for 10-15 minutes. One ml of serum was accurately obtained. Serum samples were obtained twice and placed into a fully automatic dual probe radioimmunity gamma counter to measure the radioactive counts for 60 seconds [13]. After determining the conversion relationship between the ECT count and the count of the dual probe radioimmunity gamma counter, the converted data is plugged into the equation below. GFR of TPS was calculated, and was expressed by the standardized body surface area (1.73 m^2), which was recorded as tGFR.

GFR = {[Dln (P1/P2)]/(T1-T2)} exp {[(T1lnP2) - (T2-lnP1)]/(T2-T1)}

In the above formula: D refers to the radioactive counts injected; T1 and T2 refer to two and four hours after the injection of the radioactive marker, respectively; P1 and P2 are the plasma radioactive counts at T1 and T2. The unit used for P1, P2 and D was cpm/ml, while unit used for T1 and T2 is minutes.

Statistical methods: Statistical analysis was conducted using statistical software SPSS 13.0. Count data was compared by Chi-square test, and measurement data was expressed as mean ± standard deviation (x±SD). A normal distribution-test for data was conducted. If data presented a normal distribution, a comparison within the group was conducted using paired *t*-test, and a comparison between groups was conducted using grouped *t*-test. If data were not in conformity with normal distribution, rank-sum test was used. Pearson's correlation analysis was used to analyze the relationship between the two GFRs obtained by the two methods within each group. *P*<0.05 was considered statistically significant.

Results

*General data and clinical data in each group (**Table 1**)*

Differences in number, gender ratio and height among groups were not statistically significant (*P*>0.05). However, age, weight and UAER in the DN1, DN2 and DN3 groups differed when compared with the DN0 group; and the differences were statistically significant (*P*<0.05).

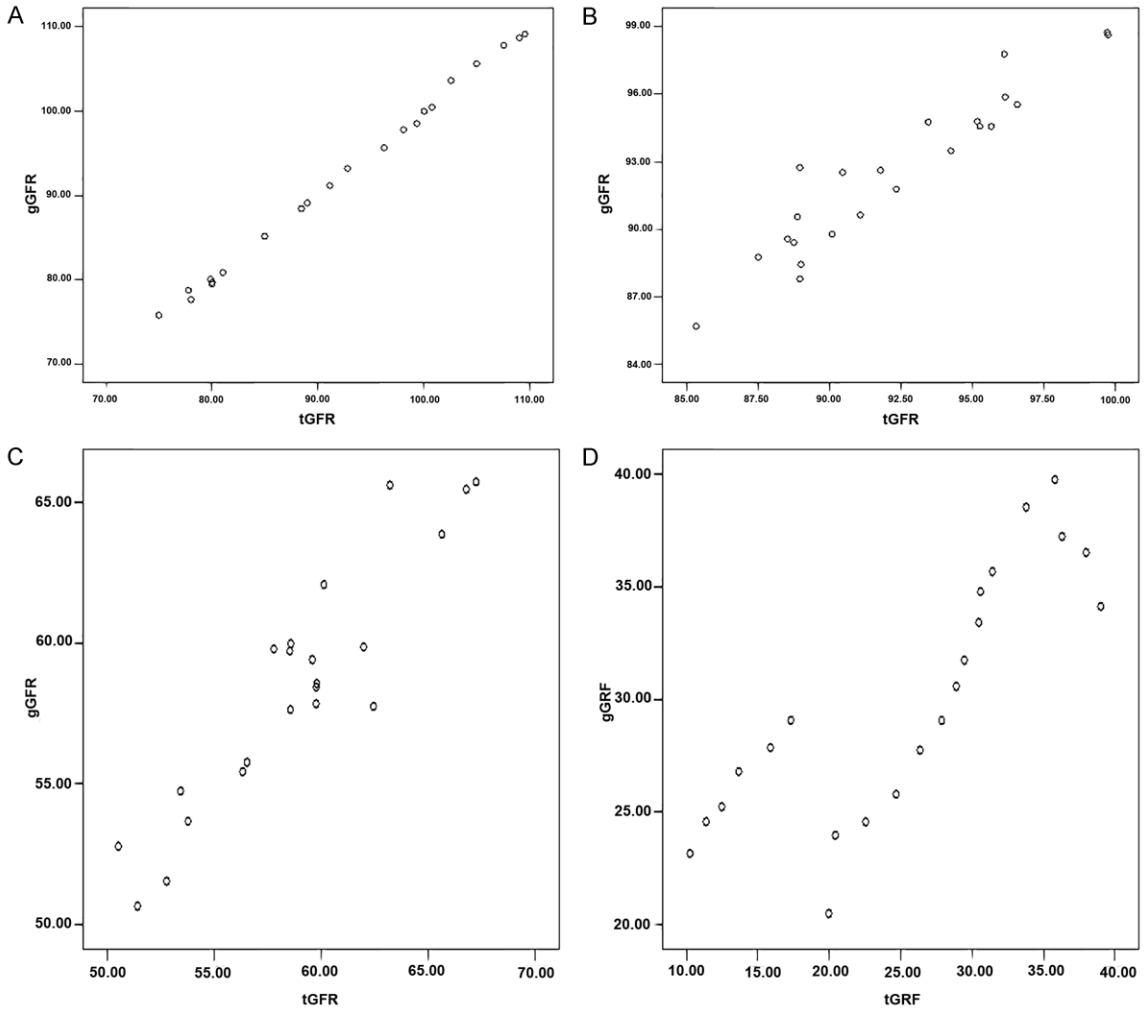

Figure 1. Scatter plot and regression equation of GFR (gGFR and tGFR, determined using RDI and TPS).

Correlation analysis between gGFR and tGFR in each group

Differences between gGFR (by RDI) and tGFRs (by TPS) in the DN0, DN1 and DN2 groups were not statistically significant ($P>0.05$). Difference between gGFR and tGFR in the DN3 group was statistically significant ($P<0.05$). Correlation coefficients between gGFR and tGFR in the DN0, DN1, DN2 and DN3 groups were 0.999, 0.947, 0.925 and 0.941, respectively; and all exhibited a significant positive correlation ($P<0.05$). There were marked differences in GFR measurements between the two methods in the DN1, DN2 and DN3 groups, compared with the DN0 group; and the differences were all statistically significant ($P<0.05$, **Table 2**). Scatter plot and regression equation of gGFR and tGFR in each group are shown in **Figure 1**.

Discussion

Diabetic renal damage may occur in the early stage of diabetes, and its progression course to DN is approximately 10 years. In terms of age, weight and UAER, DN patients that met the selection criteria in this study differed from controls; and the differences were statistically significant ($P<0.05$). In DN patients, body weight decreases with the progression of the disease, while UAER first increases and finally decreases [14]. In the early stage of diabetic renal damage, GFR increases in response to stress, and the kidney remains in a high-pressure perfusion status [15]. When this progress to early diabetic nephropathy, GFR may be slightly higher or equal to normal; and thereafter, GFR gradually reduces with the progress of the nephropathy [16]. Previously, the recog-

nized gold standard for the determination of GFR was inulin clearance; but its tedious operation and expensive cost limits its application in clinic. At a later date, it was found that more than 95% of ^{99m}TC-DTPA was filtrated by glomeruli without renal tubular secretion and reabsorption [17], and the plasma clearance of ^{99m}TC-DTPA has a very good correlation with inulin clearance. In addition, ^{99m}TC-DTPA is cheaper and easier to prepare; thus, ^{99m}TC-DTPA has become a substitute agent of inulin in clinical and scientific studies. The plasma clearance of ^{99m}TC-DTPA can be determined by RDI and the TPS method [18].

The standard procedure for determining GFR by RDI is the Gates' method, because it has many influence factors such as the patient's own condition, the injection of radioactive drugs, the protein binding rate of radioactive drugs, the outline of the renal regions-of-interest (ROI), the background chosen, kidney depth and patient's age. Hence, the accuracy of RDI may not be very good [19]. Furthermore, the influence factors of the TPS method are few. In addition, in recent years, the TPS method has become the evaluation standard for determining GFR. A number of studies have reported that TPS was used as a standard to evaluate the accuracy of RDI. For example, Peng Xie, jian-min Huang and Liping Pan specifically proposed in their study that in DN patients, RDI remained not an inadequate substitute for TPS, and the difference between the GFRs determined by these two methods was statistically significant, especially under the condition that GFR was <30 ml/min (after standardization of the body surface area). Hong-xia Yao, Jin-shan Zhang, and Shu-xia Wang *et al.* also put forward a similar point of view; and considered that the consistency of RDI and TPS in patients with renal function impairment at different stages was not good.

In this study, for the GFRs in the 22 healthy controls, 23 early DN patients, 23 clinical DN patients and 23 patients with uremia caused by diabetes, using both RDI and TPS, differences between gGFR and tGFR in the DN0, DN1 and DN2 group was not statistically significant (P>0.05). However, the difference in uremia group was statistically significant (P<0.05). These results revealed that when GFR was relatively low, gGFR determined by RDI was significantly higher than tGFR determined by TPS. Furthermore, the accuracy of gGFR was bad and was not adequate to be applied for renal failure patients with low GFR, which is consistent with results of studies conducted by Liang Su *et al*. It was also found that in healthy controls, gGFR and tGFR had a good correlation; and the correlation coefficient was 0.999 (P<0.05). In DN patients, although the correlation between gGFR and tGFR was also very good (P<0.05), the correlation coefficient was small; hence, the correlation was relatively poor. In the uremia group, the correlation coefficient was the smallest, suggesting that the correlation of GFRs determined by the two methods in the uremia group was not very good. From the scatter plot of gGFR and tGFR in each group, an obvious linear relationship between gGFR and tGFR was clearly revealed. However, the linear relationship in DN patients was poor, compared with the control group. GFRs determined by the two methods in early DN patients, clinical DN patients and uremia patients were all significantly lower than that of healthy controls; suggesting that RDI and TPS can be used in the diagnosis of DN, and sensitively reflect changes in GFR. In the diagnosis of uremia, the accuracy of RDI was relatively poor, but its determination of GFR was higher. These can easily cause a missed diagnosis. In this study, the GFR of different populations such as healthy controls, early DN patients, clinical DN patients and uremia patients were determined using RDI and TPS. The application significance of determining GFR by RDI and TPS in different populations was evaluated. However, in this study, only the total GFR of double kidneys was compared; and the partial GFR of an individual kidney was not compared. Total GFR can be influenced by many factors such as stress compensatory responses and hormone effects, and all these can easily affect the accuracy of the evaluation.

In summary, both RDI and TPS can be used for the diagnosis of DN. The early detection of GFR changes are sensitive in early DN and clinical DN. However, RDI exhibited poor accuracy and low sensitivity in the diagnosis of DN in the uremic phase with low sensitivity. Hence, medical history and other examination methods should be concurrently applied.

Disclosure of conflict of interest

None.

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