AASCIT American Association for Science and Technology

Detection of early stage renal disease by elevation of certain low molecular Weight proteins in urine of diabetes patients

Nazarian Zahra[1], Mohiti Ardekani Javad[2], Nazarian Aboalfazl[3], *

[1]International branch sahhid beheshti Medical Science Un. Tehran, Iran
[2]Clinical Biochemistry. Dept. Yazd Medical Science Un. Yazd, Iran
[3]Clinical Biochemistry. Dept. Zanjan Medical Science Un. Zanjan, Iran

Email address

nazarian@zums.ac.ir (N. Aboalfazl)

Citation

Abstract

Background: Early detection of kidney failure is critical since it is the first message for the advanced process of nephropathy. Objectives: this study is stated the correlation between glycemic control and urinary certain low molecular weight proteins (LMWP); retinol binding protein (RBP) and nerve growth factor receptor (NGFR). Methods: 30 patients with diabetes mellitus type 2 between age of 30-70 with a normal serum creatinine (<1.3mg/dl) and 30 healthy individuals in same age and sex group were randomly selected. Results: There was significant increase in RBP (P< 0.001), NGFR (P<0.005) and HbA1c (glycosylated hemoglobin) (P<0.001) in diabetics patients as compared to control group, and obtained significant correlation between LMWP and HbA1c. This study was showed negative correlation between conventional markers for subclinical nephropathy like: serum and urine creatinine, urine albumin, creatinine clearance and 24h urine protein with LMWP. Conclusion: certain tubular LMWP may alert as early stage biomarkers for predicting of renal dysfunction.

1. Introduction

Diabetes mellitus is a common health problem affecting all ages. Nephropathy is a critical micro vascular diabetic complication in type 2 diabetics leading to end stage of kidney disease (1, 2). About 30-40% of diabetic individuals develops end stage of renal and requires either dialysis or renal transplantation (3). Early detection of renal failure is critical for decrease of mortality and morbidity (4). The conventional procedure of nephropathy consists either increasing of micro albumin and serum creatinine or decreasing of creatinine clearance (5). The novel studies have confirmed that low molecular weight proteins from tubular components may excrete and elevate in renal diabetic complication in urine (6). In fact, tubule involvement may precede glomerulus involvement. Since certain of these tubular proteins are detectable before release of micro albumin and elevating of serum creatinine (7). Certain low molecular weight proteins (8) ; RBP (9,10) and NGFR(11) are not detectable or excrete at very low concentration in healthy controls but may elevate in diabetics thus determination of these biomarkers in

various stage of diabetic nephropathy displays the tubular involvements (12). LMWP have been studied in urine of diabetics and its function confirmed as early detector of diabetic nephropathy (13).

2. Materials and Methods

2.1. Design and Population

In this experimental study, prior to the initiation of the experiment, permission was obtained from the Ethical Committee of Zanjan University of Medical Science. All participants were informed about the aim of the study and a written consent was obtained from them. Following conformation of diabetes, in accordance with approved guidelines released by World Health Organization (WHO)(14), 30 patients (17 female and 13 male), between the ages of 30 - 70, referring to diabetic clinic of Zanjan Medical University from April-December 2007, were randomly selected. Zanjan City is situated in northwest part of Iran with population of nearly 486495 people, in which Diabetes clinic is actively engaged in this issue.

Socio-demographic characteristics were obtained in privacy using structured questionnaires which was designed on socio-demographic characteristics including questions on age, sex, smoking habit, intensity of physical activity, duration of disease and etc.

All participants were selected from diabetic patients and healthy individuals. Patients with any diseases other than diabetes, which may affect kidney functions, were excluded from this study. As it is shown in table 1, participants were categorized in three groups. GroupA, patients with albuminuria, group B, patients with normoalbuminuria and group C, healthy individuals, as control group, who match in sex and age with groups A & B.

2.2. Sampling Strategy

Following 12h fasting, venous blood samples were collected into two test tubes. First tube without anticoagulant to obtain serum and second tube contained K3 ethylendiamine tetra acetate (EDTA) to obtain whole blood. In order to minimize periodical variation in protein excretion, a morning urine sample was collected from each individual, in a container containing sodium azid as antiseptic. Urine and blood specimens were immediately centrifuged (2,000g for 10 min). All collected serums were frozen at -30c for subsequent biochemical analysis.

2.3. Chemicals

All chemicals were obtained from the high grade quality. All other chemicals were purchased from Merck GmbH or Sigma Company from Germany.

2.3.1. Laboratory Evaluation

All urine specimens centrifuged, and their supernatant mixed with ammonium sulfate (40%) for 30 min. to precipitate all proteins, then concentrated with dialyzing tube against dry sugar overnight, the proteins were purified with chromatography (DEAE-cell) in Tris -HCL (pH=8), then eluted by gradient of NaCl (0.3-0.5M) and proteins detected with UV spectrophotometer at 280 nm, and by electrophoresis (SDS-PAGE) at 40 mA in comparison with standard markers.

Serum glucose, serum and urine creatinine, micro albumin, urine protein were measured by commercially assay kits (Pars Azmoon Co Ltd Iran). Glycosylated hemoglobin (HbA1c) was measured in whole blood by using liquid column chromatography (Bio-Science Co Ltd Spain). HbA1c in non diabetic, good glycemic control and poor glycemic control were set to be <6 %, < 7 % and > 8%, respectively.

Retinol Binding Protein (RBP) and Nerve Growth Factor Receptor (NGFR) determined by Elisa kit (Sigma Co Ltd Germany) by elisa reader (stat fax 2000). NGFR level also confirmed with radial immune diffusion technique in agar medium containing human anti-NGFR (Sigma Co Ltd Germany).

Creatinine clearance was calculated with reference to age, sex, and serum and urine creatinine using standard formula) [(140-Age) ×Weight (Kg)/72×s creatinine (mg/dl)] method. Normo albuminuria and Albuminuria were defined as below 30 mg/g creatinine and 30- 300 mg/g creatinine, respectively.

2.4. Statistical Analysis

All data were calculated as mean ± standard error of the mean (SEM). The difference between studies groups were obtained with student's unpaired t-test. A P value of 0.05 denoted the presence of statistical significance.

3. Results

The significant differences were not observed in different ages and sexes between groups. A significant increase for diabetic patient was obtained in Fasting Blood Sugar (FBS) (P<0.05), 2hour post prandial Blood Sugar (2hpp BS) (P<0.05), HbA1c (P<0.001), micro albumin (P<0.001), creatinine clearance (P<0.01), RBP (P<0.001) and NGFR (P<0.005) in compared with control group (table 1).

In this study, diabetic patients with micro albuminuria showed a significant increase in urinary albumin (P<0.01), RBP (P<0.001), NGFR (P<0.005) as compare to diabetic patient with normo albuminria. Following biochemical analysis, the mean analysts values for studying groups are showed in table 1: FBS, 2hppBS, HbA1c, U protein, U albumin, S Creatinine, U Creatinine, Creatinnine Clearance, RBP, NGFR for Group A are significantly higher than for group B and group C respectively. Following column chromatography (DEAE-Cell), the absorbance of proteins were 0.14, by wash and 0.25 by elution methods (Fig. 2). The Purified LMWP were dissociated by SDS-PAGE Electrophoresis in 12.5% gel with tris-HCl (pH=8) and

Acetate (pH=5) in 350 volt and for final evaluation, obtained results were compared with Standard Protein Markers(sigma).The anti-NGFR (sigma) were interacted in dark zone around well containing purified LMWP (Figure 3), as it is shown in table.1, the mean RBP and NGFR

levels of patients with albuminuria were more than those with normo albuminuria and control group. HbA1c value in non diabetic, good and poor glycemic control was set as 5-6.5 %, 6.6-8 % and > 8%, respectively.

Table 1. *Biochemical analysis for different groups in current study, including control group Group C and Diabetic patients (Groups A & B)*

|		**Diabetic Patients**		
	Control C	**All patients**	**Albuminuria A**	**Normo-albuminuria B**
(Gender) M/F	15/15	13/17	3/8	10/9
Age (years)	55.2 ± 3.6	56.7 ±1.8	54.1 ± 2.3	59.2 ± 2.12
FBS (mg/dl)	85.5±3	187±10	225±12	149±11
2hpp BS (mg/dl)	90 ± 10	192± 11	204±12	179±10
HbA1c (%)	5.2 ± 0.16	8.5 ± 0.2	8.5± 0.4	8.0± 0.3
Uprotein (mg/24h)	230± 4	362± 4	358 ± 5	185±4
Ualbumin (mg/24h)	110± 2	216.5± 2	308 ± 3	125 ± 2
SCreatinine(mg/dl)	0.88± 0.04	1.18± 0.05	1.15± 0.04	1.05± 0.04
UCreatinine (g/l)	0.328± 0.03	0.316± 0.03	0.308± 0.04	0.321± 0.035
Creatinine Clr*. (Ml/min)	95.20 ± 2.5	76.38±3.1	57.47 ± 2.5	95.17± 3.14
RBP (μg/l)	0.38± 3	0.58± 2.8	0.77 ± 2	0.42± 3
NGFR (μg/mgCr)	0.43±0.5	0.68± 0.6	0.81± 0.4	0.53 ± 0.65

Abreviations: U =Urine, S=Serum, Clr=Clearance,RBP=retinol binding protein, NGFR=nerve growth factor receptor *Cockcroft and Gault Formula* Calculated creatinine Clearance [(140-Age×Weight(Kg)/72×screatinine For females, subtract 15% (or multiply by 0.085).
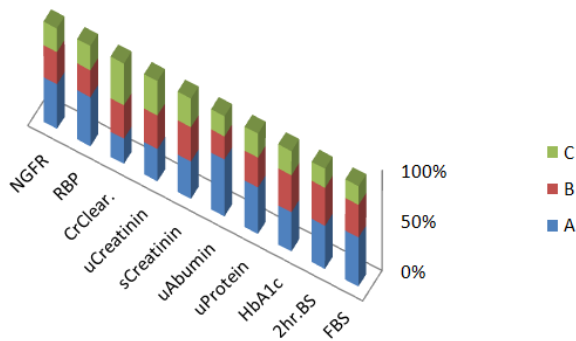
Fig 1. *Comparison between different markers for effect of early phase nephropathy diagnosis. Group: Patients (A, B), Controls(C).*
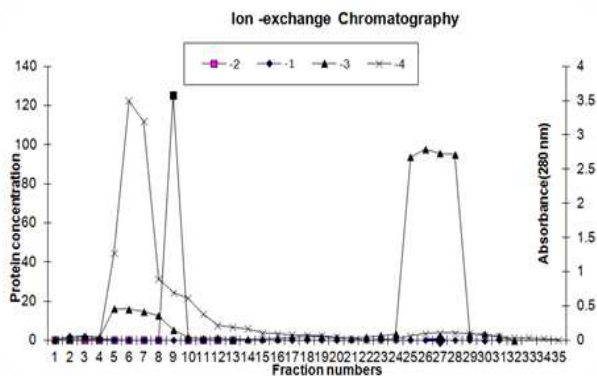
Fig 2. *Purification plot of different samples with Ion-Echange Chromatography (DEAE-Cell): 1: wash ,2-4: elution with 0.3-0.5M NaCl.*
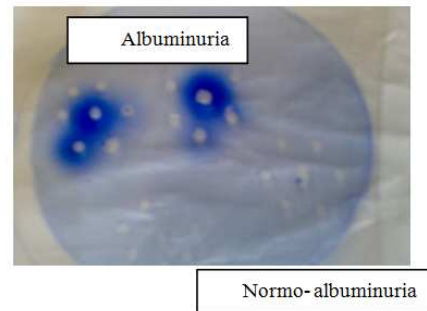
Fig 3. *Simple Radial Diffusion, NGFR in Albuminuria patient(Group A) formed positive dark zone with anti-NGFR which embeded into agarose.Control Group(C) & normo-albuminuria (B) displayed negative response with Anti-NGFR.*

The sensitivity of tests for biochemical parameters; serum and urine creatinine, creatinine clearance, urine albumin, NGFR and RBP were 27%, 29%, 52%, 30%, 85% and 76% respectively, and the specificity of test for serum and urine creatinine, creatinine clearance, urine albumin, NGFR and RBP were 26%, 28%, 53%, 35%, 90 and 70% respectively.

4. Discussion

This study was showed an elevation in two biomarker of low molecular proteins; RBP and NGFR in type 2 diabetes as diagnostic signal in early stages of tubular dysfunction. Other workers also stated that detection of kidney tubular protein may precede the glumerular dysfunction (13-15). These LMWP are released even before excretion of micro albuminuria (15).Some workers believed that urinary

LMWP have been recommended as helpful messages for assessment of changes in proximal tubular action before any other markers as proteinuria and rising of serum creatinine (16). In this study was showed that urine excretion or RBP and NGFR were higher in diabetic patients (P< 0.001) than healthy controls which confirmed an increase in glumerular excretion or decrease reabsorbtion in proximal tubules. This work showed similar results as Chue et al (17) stated that RBP was higher in diabetic persons than in control. Some research workers (18, 19) suggested that RBP a LMWP is released by glumeruli and reabsorbed and catalyzed by proximal tubules. The elevation of urine RBP occurs with altered tubular function and then tubular reabsorbtion is overload (20). The induction of NGFR excretion was suggested to correlate with RBP. This study was done to observe renal tubular function in type 2 diabetes by determination of RBP and NGFR and their correlation with metabolic factor like as duration of diabetes, glycemic control (HBA1c) , creatinine clearance and urine protein as tubular dysfunction markers for early detection of sub clinical nephropathy(21,22).

References

[1]   Waqar Kashif, Nauman Siddhqi, Erhan Dincer H, Ayse P. Dincer, Sheldon Hirsch. Proteinuria: How to evaluate an important finding.   Cleveland Clinic Journal of Medicine.2003; 70(6):535-547.

[2]   American Diabetes Association. Type 2 diabetes in children and adolescents. Diabetes Care 2000; 23(3):381-389.

[3]   Varma PP, Murthy S N, Hooda A K. Proteinuria- Long Term Complications. Medicine Update2008; 18:156-168.

[4]   Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. Diabetes Res Clin Pract 2003;61(1):29-37.

[5]   Gul E. Rana et al. Clinical Significance and prognostic value of low molecular weight "tubular" proteins, a-1 acid glycoprotein in diabetes. Pak J Physiol2009; 5(1):34-36.

[6]   Manal A.F et al. Estimation of renal tubular markers for predicting early stage diabetic nephropathy in Egyptian children with type I diabetes mellitus. . Res.J. Medcine & Medical Sciences2009; 4(2):207-211

[7]   Schlatzer D, Maahs DM, Chance MR, Dazard JE, Li X, Hazlett F, Rewers M, Snell-Bergeon JK - Novel urinary protein biomarkers predicting the development of microalbuminuria and renal function decline in type 1 diabetes. Diabetes Care. 2012; 35(3):549-55.

[8]   Hahn WH, Suh JS. Linkage and association study of neurotrophins and their receptors as novel susceptibility genes for childhood IgA nephropathy. Pediatr Res. 2011; 69(4):299-305.

[9]   Ayatse JO. Human retinol-binding protein: its relationship to renal function in renal diseases. West Afr J Med. 1991; 10 (3-4):226-31.

[10]   Kirsztajn GM, Nishida SK, Silva MS, et al: Urinary retinol-binding protein as a prognostic marker in glomerulopathies. Nephron 2002; 90 (4):424-431

[11]   Luigi Aloe, Simona Rossi, Luigi Manni. Altered expression of nerve growth factor and Its receptors in the kidneys of diabetic rats. J Nephrol 2011; 24(06): 798-805.

[12]   Antonucci MT, Bonofiglio R, Papalia T, Caruso F, Caroleo MC, Mancuso D, Aloe L. Nerve growth factor and its monocyte receptors are affected in kidney disease.Nepron Clin Prac 2009; 111(1):21-8.

[13]   Fagot-Campagna A, Pettitt D, Engelgau M, Burrows N, Geiss L, Valdez R, et al. Type 2 diabetes among North American children and adolescents: an epidemiologic review and a public health perspective. J Pediatr 2000;136(5):664-72.

[14]   Ching-JE hong et al. Urinary microglobulin as a marker of nephropathy in type II diabetic asian subject in Singapoor. Diabetic Care2003; 26:338-342.

[15]   Sariola, H., et al. Dependence of kidney morphogenesis on the expression of nerve growth factor receptor. Science1991; 254:571-573.

[16]   Nosadini R, Tonolo G. Role of oxidized low density lipoproteins and free fatty acids in the pathogenesis of glomerulopathy and tubulointerstitial lesions in type 2 diabetes. Nutr Metab Cardiovasc Dis. 2011; 21(2):79-85.

[17]   Chu CH, Lam HC, Lee JK, Lu CC, Sun CC, Cheng HJ, Wang MC, Chuang MJ. Elevated serum retinol-binding protein 4 concentrations are associated with chronic kidney disease but not with the higher carotid intima-media thickness in type 2 diabetic subjects. Endocr J. 2011;58 (10):841-7.

[18]   Mona A. K. Salema, . Mohamed M. K. et al Urinary excretion of n-acetyl (- -glucosaminidase and retinol-binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus Pediatric Diabetes 2002: 3: 37–41.

[19]   John G. Lewis , Brett I. Shand , Peter A. Elder , Russell S. Scott Plasma retinol-binding protein is unlikely to be a useful marker of insulin resistance diabetes research and clinical Practice2008; 8 0 : e 1 3– e1 5

[20]   Najla Shore, Rukhshan Khurshid*, Mahjabeen Saleem** 1Alpha-1microglobulin: A marker forearly detection of tubular disorders in diabetic nephropathy. J Ayub Med Coll Abbottabad 2010;22(4):53-5

[21]   Dhruv K,Fraington S K.The tubule-interstitium in early diabetic nephropathy.www.IJddc.com. 2010; 59:141-147

[22]   Kirsztajn GM, Nishida SK, Silva MS, et al: Urinary retinol-binding protein as a prognostic marker in glomerulopathies. Nephron 2002 Apr;90 (4):424-431