Prediction of Chronic Kidney Disease in Type 2 Diabetes Mellitus patients

Hadeel Luay kareem[1], Shaymaa Z. Nada[2], Farqad A. Abdulhasan[3]
[1]Department of Medical Laboratory Techniques, Al-Mustaqbal University College, 51001 Hillah, Babylon, Iraq., Email: Hadeel.luay@mustaqbal-college.edu.iq
[2]University of Kerbala– College of Medicine, Iraq
[3]University of Ahl Al Bayt/Iraq

Abstract

Background: Chronic kidney disease CKD is a common condition that closely related to the Type 2 Diabetes Mellitus T2DM patients. thus, one of the wares complication that causing morbidity and mortality. According torelationshipofCKDandT2DM there is some cytokines that involved in development of both diseases. ZingAlpha2Glycoprotein (ZAG)is a novel cytokines synthesis and releasing from liver in certain disease as indicator for progression outcomes. Aim of this study: The goal of this study was to estimates erumlevelso fZAGin patientswithT2DM including with and without CKD. Subject and Method: we use ELISA assay to measurements serum ZAG in this studycompressing61T2DMincluding 20haveCKDand control subjects60. Result: Serum ZAG significantly increased in patient with CKD (106.71±15.923 ng/ml),comparingwith T2DM(88.14±9.610ng/ml)ascompering with control subject (55.1± 12.108 ng/ml) and (P-value < 0.05). Furthermore, closely correlated with Gender, BMI duration of T2DM, FBL, HbA1c, lipidprofile and liver enzyme (ALT,AST). Conclusion: Serum concentrations of ZAG useful predictor biomarker for early diagnostic of diabetic nephropathy and it may involve in predicting theCKD outcome ofT2DM. Abbreviations: Chronic kidney disease CKD, Type 2 Diabetes Mellitus T2DM, Zing Alpha 2 Glycoprotein ZAG, Fasting Blood Sugar FBS, Body Mass Index BMI.

Keywords case-control ● Zing alpha2 glycoprotein ZAG●T2DM●chronic kidney disease CKD●ELISA Assa

1. Introduction

DiabetesMellitus,referredtoas"diabetes"isachronicdiseas eassociatedwith abnormally high levels of the glucose in the blood, major complications of diabetes are damage the eyes, nerves, heart and kidney the last leading to Chronic kidney disease(CKD). This a general term that including heterogeneous disorders, probably kidney damage by certain disease or accidentally or may decreased glomerular filtration rate(GFR).This definition has been widely use and unchanging since2002 [1].

Diabetic nephropathy affects all of the cellular components in the glomeruli and renal tubular interstitial, However, some patients with diabetes can experience a decrease in eGFR and may progress to end stage renal disease without having any significant albuminuria.

Interestingly,indiabeticnephropathy,kidneyfunctioncorre latesgreaterwith the degree of tubulointerstitial injury rather than with glomerular injury hence, biomarkersthatcorrelateswithtubulardamagehavebeens uggestedbyvariousstudy[2].Giventhis,immunohistochemi cally(IHC)analyseshavedemonstratedthatZAGisexpressed mainlyinthetubulesofthehumankidney [3]. This study suggested that the concentrations of ZAG might increase earlier indicator in the progression of diabetic nephropathy, before microalbuminuria becomes apparent. These additional proteins can be used as a indicator for early diagnostic for kidney damage in T2DM [4].

Zinc a2-glycoprotein (ZAG) is a human protein firstly isolated from human bloodplasmain1961.Namederivedfromitselectrophoreticm obilityinthe alpha2regionandbecauseitcanbindwithzincionsandprecipit atedduring isolation [3, 5, 6]. Molecular mass about 40-kDa single-chain polypeptide [7]. Consists of 276 amino acid and has a molecular weight of 31,889 [8, 9]. It is considered as a novel adipokine isolated from human blood plasma, later found in other body fluids, such as saliva, sweat, breast milk, seminal, plasma, cerebrospinal and urine [3, 5, 10, 11].

ZAG has been newly submit as an adipokine contributory in body weight control, ZAG levels in serum are raised in chronic hemodialysis patients as compared with control [12]. Meanwhile, explain that ZAG is not only high in chronic hemodialysis patients but have potentially raise during the early phase of acute kidney injury [4]. ZAG also has another effect suggested that promoted lipid in dissolution adipocytes and causes the great fat losses related withdifferentprogressmalignant.YuanWangetal.firstsugg estedthatZAG might be a beneficial, biomarker for early indicator of diabetic nephropathy in patients with T2DM [13]. ZAG have novel role in regulation of fibrosis. Chronicrenaldiseasealmostresultsfromdevelopingofkidne yfibrosisthelast will causing a damage in kidney cell (acute kidney injury). Gradually, at the end loss the renal function (kidney failure). ZAG regulation of kidney and heart fibrosis.

Aim of this study: is to link the relationship between biomarkers serum ZAG and type2diabetes mellitus with chronic kidney disease and suggested this protein as indicator for early diagnostic of kidney damage.

2. Materials and Methods

Samples Collection

doi.org/10.31838/hiv22.02.234 Received: 05.05.22, Revised: 06.06.22, Accepted: 20.08.22

All participants were recruited at the department of Al-Husain Teaching Hospital/ Karbala and collected from November2018tillApril,2019. Were randomly selected from the patients attending the Diabetic Consultation Unit and dialysis Unit at the Hospital, patients with diagnosis of DM and/ or DM with CKD was including. The medical history of each patient was taken regarding age, gender, diabetes mellitus (DM), type of treatment, history of renal disease, history of any other diseases. Additionally, patients with chronic liver disease, patient with thyroid problem (hyper or hypo thyroidism), patient with malignancies, all subjects ages up70 less than 30, CKDoutofDMT2,Cardiovasculardiseases include coronary artery disease, peripheral vascular disease and stroke, and the infections and emergency patients were excluded.

The present study approved by Ethical Committees, which include: Committee of College of Medicine in University of Kerbala, committee of Diabetes and Endocrinology Center in Al-Hussein Teaching Hospital at Al-Hussein Medical City, Karbala Health Directorate/Holly Karbala-Iraq, and the Center Habib bin Mudhaher Al-Asadi for dialysis in the same hospital.

The study design is a case control study, was executed on (n=60) control and (n=61) patients, age ranged between (30-70) years (males 40 and females 81). The selection of the patients depends on a number of criteria the practical side of the study was performed at the. Were randomly selected from the patients attending the Diabetic Consultation Unit and dialysis Unit at the Hospital, Questionnaires were designed to obtain the information of control and cases group.

Blood samples were collected from each case after being fast for10-12hours.The collected blood volume is 5 milliliters, which withdrawn by disposable syringes in the sitting position. The collected blood stored in two clean and sterilized tubes: one is gel tubes (contain a special gel that separates blood cells from serum to cause blood to clot quickly), and the other one is EDTA tube for HBA1c. The samples were collected between 08.00-12.30 am. Blood was allowed to clot at 37°C for 10-15 minutes and then centrifuged at 2000xg for approximately10-15minutes.After, theserumisdividedintotwopartsand storedat-20°C.Thecollectedserumfrompatientsandcontrolswereusedfor the measurements of the following parameters: ZAG, Fasting blood sugar, lipid profile (Total cholesterol, LDL-C, VLDL-C, HDL-C, and TG). Serum Creatinine, Urea and liver enzymes (GOT, GPT). The study was approved by the local ethics committee, and all subjects gave written informed consent before taking part in the study.

Study Measurements

Clinical estimation

Standardized protocols were used to measure height and body weight in all participants in order to calculated Body mass index (BMI) by equation, the calculation done by weight(kg) divided by square height (m2) according to standardized formulas. The GFR was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) Study formula. This formula it is likely to be more accurate as it may adjust for variations in Creatinine measurements. The eGFR value was calculated by using age, sex, serum creatinine (S. Cr) and race in population different races but this study conducted on one race. using the following MDRD equation [14]:

$$eGFR(ml/min/1.73m2) = 186(S.\ Cr) -1.154*(age)-0.203*(0.742iffemale) *(1.21ifblackrace)$$

Estimate of serum ZAG levels.

Serum ZAG levels were measuredaccordingtothemanufacturer'sinstructions (ElabScience, China). Therangeofdetectionwas93.75–6000ng/mL,withanintra-assay coefficient of variance (CV) < 10%. No significant cross reactivity or interference was observed.

3. Statistical Analysis

Statistical Analysis bySocial Science software (SPSS24IBM, Armonk,USA) was used to explain the results by mean ± standards deviation(SD). Differences between patients and control groups were performed using (one-way ANOVA) test and T-test. The calculation of correlations between all variables were estimated using Pearson's correlation coefficient(r).

4. Results

The clinical characteristics of all subjects were included(n=60)patientsofT2DM,(n=20) patients with CKD(10 males and 10 females; mean ± SD age, 52.70±11.567years) and 41patients T2DM without CKD(n=41) (10 males and31females; mean±SD age,50.12±11.533years), (n=60)control apparently healthy (20 males and 40 females; mean ± SD age, 48.83 ± 10.320 years). Table 1.illustreated the mean values of all parameters that measured in this study.

Table1:ClinicalCharacteristicsofT2DMpatientofbothwithandwithoutCKD

Parameters	T2DM Mean ± SD(N=61)		Control Mean ± SD (N=60)	p- value
	CKD(N=20)	T2DM(N=41)		
Age (30-70) year	52.70 ± 11.567	50.12 ± 11.53	48.83 ± 10.32	>0.05
Gender(male/female)	10/10	10/31	20/40	<0.05
BMI(kg/m2)	28.21 ± 7.07	29.02 ± 6.61	29.186.08	>0.05
Duration T2DM(year)	7.60 ± 6.747	7.15 ± 5.79	0.00 ± 0.000	<0.05
eGFR(mL/min/1.73m2)	52.05 ± 37.40	121 ± 44.5	108.4 ± 20.14	<0.05

S.Urea(mg/dl)	82.68 ± 62.446	24.95 ± 7.47	28.86 ± 7.753	<0.05
S.Cre (mg/dl)	2.95 ± 3.413	0.80 ± 0.975	0.69 ± 0.145	<0.05
FBS(mg/dl)	227.2 ± 106.95	239.62 ± 90.99	135.99 ± 69.52	<0.05
HbA1c(mg/dl)	9.03 ± 2.926	9.91 ± 2.55	5.61 ± 0.661	<0.05
Cholesterol(mg/dl)	189.91 ± 42.16	193.19 ± 51.92	197.50 ± 34.41	>0.05
HDL-C(mg/dl)	40.71 ± 8.959	45.37 ± 15.57	42.45 ± 7.583	>0.05
LDL-C(mg/dl)	126.92 ± 39.59	135.79 ± 44.25	142.74 ± 31.2	>0.05
TG(mg/dl)	189.11 ± 92.35	209.3 ± 119.65	169.79 ± 77.20	>0.05
ALT(mg/dl)	26.99 ± 23.208	21.35 ± 11.05	20.53 ± 10.61	>0.05
AST(mg/dl)	31.20 ± 30.841	21.86 ± 11.08	22.09 ± 16.22	>0.05
ZAG(ng/ml)	106.71 ± 15.923	88.14 ± 9.610	55.1 ± 12.108	<0.05

SD: Standard deviation, BMI: body mass index, eGFR: estimated Glomerular Filtration Rate, S.cre: serum creatinine, S.urea:serum urea, FBS: Fasting Blood Sugar, HbA1c: Hemoglobin A1c, HDL-C: High Density Lipoprotein, LDL-C: Low Density Lipoprotein, TG: Triglycerides, ALT(SGOT): Ala- nine Aminotransferase, AST (SGPT): Aspartate Aminotransferase, ZAG: Zing Alpha 2 Glycoprotein.

according to the presented data which shown in Table 1. The results indicated a significant differences between T2DM patients and control based on gender, eGFR estimated glomerular filtration rate urea, creatinine, fasting blood sugar, HbA1C, ZAG (p value < 0.05). While there are no significant in age, body mass index BMI, cholesterol, HDL-C, LDL-C, triglycerides TG, AST, ALT.

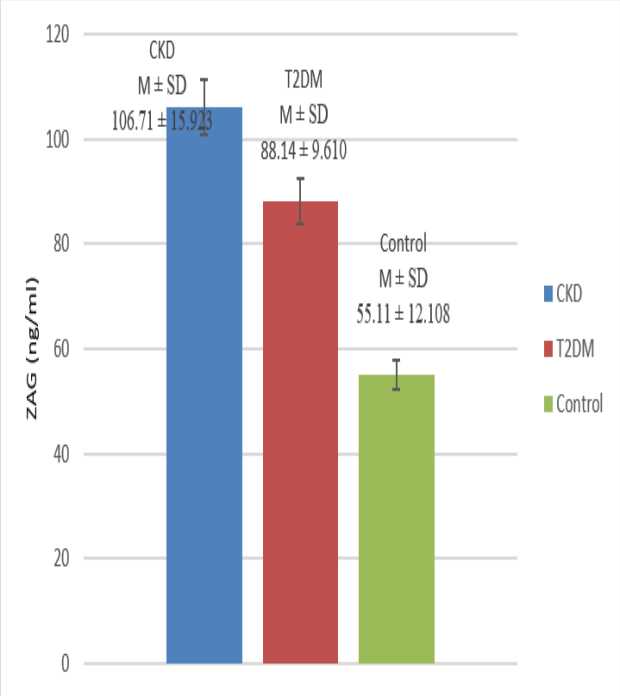

FIGURE 1: Shows mean levels of significant result of ZAG (ng/ml) increased in patients group of T2DM with and without CKD versus controls subject.

Correlations between variables were estimated using Pearson's correlation coefficient (r) and linear regression analyses as shown in Table2.

Table2:CorrelationbetweenZAGandmeasured parameters.		
Parameters	**ZAG ng/ml**	
	P-Value	**r**
Age (30-70) year	0.471	0.094
Gender(male/female)	0.005	-0.354
BMI(kg/m2)	0.128	-0.173
Duration T2DM(year)	0.214	0.161
eGFR(mL/min/1.73m2)	0.001	-0.429
Urea(mg/dl)	0.000	0.642
Creatinine (mg/dl)	0.000	0.484
FBS(mg/dl)	0.028	-0.281
HbA1c(mg/dl)	0.005	-0.357
Cholesterol(mg/dl)	0.325	-0.128
HDL(mg/dl)	0.548	-0.079
LDL(mg/dl)	0.165	-0.180
TG(mg/dl)	0.263	-0.146
ALT(mg/dl)	0.993	-0.001
AST(mg/dl)	0.566	0.075

5. Discussions

Chronickidneydisease(CKD)isacommonconditionthatclos elyrelatedtothe diabetic patient, thus, one of the wares complication that causing morbidity and mortality. In the last few decades, the expectations that the number of patients with severe diabetic kidney disease increased rapidly to reach 552 million people by the end of 2030 [15]. AccordingtorelationshipofCKDandT2DM,thereis some cytokines that involved in development both of them. Such as zincalpha 2glycoproteins ZAG, is a human protein it is considered as a novel adipokine involve in many defects [10], regulation of body weight, body fat, and glucose metabolism [16].Actually, researchers have interested in functions of ZAG because considered as a multidisciplinary functional continues to be under study such as fertilization a lipid mobilization and high expression in diverse disease such as cancer cachexia, In recent years several researchers groups have reported the relationship of this protein with T2DM with CKD, Philipp *et al*. revealed for the first time that median serum ZAG levels are almost 2-fold higher in chronic hemodialysis CD patients as compared with controls. Accordingly, he suggests that renal filtration is an important way of elimination of ZAG and that markers of renal function should be included in studies on ZAG physiology. In addition, chronic hemodialysis stays a strong independent Speculate for ZAG level in multivariate analysis, However, Philipp and coworker did not found any relationship with other parameters this is contrast to our findings [12].In this current study revealed a negative significant correlation with renal function and with FBS and negative relation with lipid profile and BMI as shown in the Table 2. On the other hand, Sorensen-Zender *et al*. findings demonstrate a useful of combined detection of serum ZAG level in

patients withacutekidneyinjuryandchronickidneydiseaseoriginally fromdiabetic nephropathy or another disease, this study illustrated that circulating protein is not increased only in CKD but also sharply elevated during acute phase of kidney injury also they found a significant relationship with serum creatinine in all patients and the high level of ZAG association sharply with high level of creatinine, and they found the higher level of this protein is also in the older and who they have many complication of CKD while they found a negative correlation with BMI and low density lipoprotein LDL with no relation with rest other lipid profile and fat body [4]. This is obviously strongly supporting our result, moreover, with a size of 40kDa ZAG is predicted to be normally filtered in the glomerulus and there after cleared by the proximal tubule through reabsorption and lysosomal degradation, however, a little disagreement with parameters may due to different population, or the author conduct his study on different stages of CKD [4].

Yuan Wang *et al*. suggested that ZAG might be a potentially useful biomarker for early diagnosis of diabetic nephropathy in patients with T2DM, they conduct their study on the patient with T2DM with and without CKD in order to detect the early stage of renal damage they found ZAG concentration in serum and urine was positively correlated with serum creatinine and eGFR but not with glucose, lipid profile as well as inflammation stator BMI [13]. This is in almost perfect agreement with our data, with a little contrary with our result, in this current study as we mentioned previously there is a significant correlation with creatinine and glucose and BMI and negative association with lipid profile as shown inTable2.

On the other hand, they revealed in diabetic nephropathy as discussed at length in their study the damage that mainly occurs in the proximal tubules rather than in the glomerular of the kidney are undergo prolonged exposure to various metabolic, they explained that renal function correlates better with the degree of tubule interstitial injury rather than the degree of glomerular lesions in CKD based on many studies [2, 13, 15, 17].

Yuan Wang *et al*. study's measured ZAG level in serum and urine, It has reported that ZAG levels elevated in urine compare to serum. Meanwhile, pathophysiological role of ZAG in renal tubules stilled obscure [18].

Steven and Michael were also demonstrated that ZAG may potentially be influential in the treatment of T2DM, experimentally they estimate the ability of recombinant ZAG to reduce metabolic features of T2DM in the ob/ob mouse model, they found recombinant-ZAG promoted a progressive loss of body weight without an effect on food or water intake. All these result supporting our findings [19].

Furthermore, Samta *et al.* report that ZAG is a one of five main additional proteins present in urine that should measure for accurate and specific prediction of diabetic kidney disease besides albuminuria test [20, 21].

Summaries of all previous result circulating ZAG is positively associated with T2DM as assessed by (FBS, HbA1c) and renal function as assessed by (S.Cr, BUrea and eGFR) as showninTable1, and patients with CKD significantly have the higher levels as shown in Figure1. Serum ZAG level also negatively correlated with facets of the lipid profile, including total Cholesterol, HDL-C, LDL-C and TG, with negative relation with ALT liver enzyme but not AST, as well as, inflammatory state as shown in Table2. However, the exact mechanisms of ZAG regulation in CKD and T2DM must be now identified through future experimental studies.

6. Conclusion

In conclusion, the biological functions of ZAG have not been fully elucidated this is need more study in the future work, serum ZAG is positively associated with increase the risk of kidney disease in patient with T2DM.

7. Acknowledgment

The authors of this work wish to acknowledge the university of Kerbala– College of Medicine and AL-Mustaqbal University college for supporting the carrying out of this research work.

References

1. CesarUgarteGil, RodrigoCarrillo, DanielaE, Kirwan. Latent tuberculosisinfectionandnoninfectiousco-morbidities:Diabetes mellitus type 2, chronic kidney disease and rheumatic arthritis. Journal of InfectiousDiseases. 2019;80.

2. Thomas M, Burns W, Cooper M. Tubular changes in early diabetic nephropathy. Advances in chronic kidney disease. 2005;12(2):177-86. https://doi.org/10.1053/j.ackd.2005.01.008

3. Tada T, Ohkubo I, Niwa M, Sasaki M, Tateyama H, Eimoto T. Immunohistochemical localization of Zn-alpha 2-glycoprotein in normal human tissues. Journal of Histochemistry & Cytochemistry. 1991;39(9):1221-6. https://doi.org/10.1177/39.9.1918940

4. Sörensen-Zender I, Beneke J, Schmidt BM, Menne J, Haller H, Schmitt R. Zinc-alpha2-glycoprotein in patients with acute and chronic kidney disease. BMC nephrology. 2013;14(1):1-6. https://doi.org/10.1186/1471-2369-14-145

5. Bürgi W, Schmid K. Preparation and properties of Zn-α2-glycoprotein of normal human plasma. Journal of Biological Chemistry. 1961;236(4):1066-74. https://doi.org/10.1016/S0021-9258(18)64243-7

6. Qu F, Ying X, Guo W, Guo Q, Chen G, Liu Y, Ding Z. The role of Zn-α2 glycoprotein in sperm motility is mediated by changes in cyclic AMP. Reproduction. 2007;134(4):569-76. Available from: https://rep.bioscientifica.com/view/journals/rep/134/4/1340569.xml

7. MohammadIKambohandRobertEFerrell. Geneticstudiesof low-abundance human plasma proteins. i. microheterogeneity of zinc-α2-glycoprotein in biological fluids. Biochemicalgenetics.24(11-12):849–57,1986.

8. Freije JP, Fueyo A, Uría J, López-Otin C. Human Zn-α2-glycoprotein cDNA cloning and expression analysis in benign and malignant breast tissues. FEBS letters.

1991;290(1-2):247-9. https://doi.org/10.1016/0014-5793(91)81271-9
9. Tomohiro Araki F, Keiichi Takagaki, HeinzHaupt,H Gerhard Schwick, W Bürgi, , Thomas Marti JS. EgonRickli,andReinhardBrossmer.Completeaminoacidsequenceofhuman plasmazn-alpha2-glycoproteinanditshomologytohistocompatibility antigens. Proceedings of the National Academy of Sciences. 1988;85(3):679–83.
10. Ceperuelo-Mallafré V, Naf S, Escoté X, Caubet E, Gomez J, Miranda M, Chacon M, Gonzalez-Clemente J, Gallart L, Gutierrez C. Circulating and adipose tissue gene expression of zinc-α2-glycoprotein in obesity: its relationship with adipokine and lipolytic gene markers in subcutaneous and visceral fat. The Journal of Clinical Endocrinology & Metabolism. 2009;94(12):5062-9. https://doi.org/10.1210/jc.2009-0764
11. Schmid WBaK. Preparation and propertiesofzn-α2-glycoproteinofnormalhumanplasma.JournalofBiological Chemistry. 1961;236(4):1066–74.
12. Philipp A, Kralisch S, Bachmann A, Lossner U, Kratzsch J, Blüher M, Stumvoll M, Fasshauer M. Serum levels of the adipokine zinc-α2-glycoprotein are increased in chronic hemodialysis. Metabolism. 2011;60(5):669-72. https://doi.org/10.1016/j.metabol.2010.06.019
13. Wang Y, Li Y-M, Zhang S, Zhao J-Y, Liu C-Y. Adipokine zinc-alpha-2-glycoprotein as a novel urinary biomarker presents earlier than microalbuminuria in diabetic nephropathy. Journal of International Medical Research. 2016;44(2):278-86. https://doi.org/10.1177/0300060515601699
14. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, Group* MoDiRDS. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Annals of internal medicine. 1999;130(6):461-70. https://doi.org/10.7326/0003-4819-130-6-199903160-00002
15. Reutens AT. Epidemiology of diabetic kidney disease. Medical Clinics. 2013;97(1):1-18. https://doi.org/10.1016/j.mcna.2012.10.001
16. Liu M, Zhu H, Dai Y, Pan H, Li N, Wang L, Yang H, Yan K, Gong F. Zinc-α2-glycoprotein is associated with obesity in Chinese people and HFD-induced obese mice. Frontiers in physiology. 2018;9:62. https://doi.org/10.3389/fphys.2018.00062
17. Tao Z, Shi A, Zhao J. Epidemiological perspectives of diabetes. Cell biochemistry and biophysics. 2015;73(1):181-5. https://doi.org/10.1007/s12013-015-0598-4
18. Schmitt R, Marlier A, Cantley LG. Zag expression during aging suppresses proliferation after kidney injury. Journal of the American Society of Nephrology. 2008;19(12):2375-83. https://doi.org/10.1681/ASN.2008010035
19. Russell ST, Tisdale MJ. Antidiabetic properties of zinc-α2-glycoprotein in ob/ob mice. Endocrinology. 2010;151(3):948-57. https://doi.org/10.1210/en.2009-0827
20. Varghese SA, Powell TB, Budisavljevic MN, Oates JC, Raymond JR, Almeida JS, Arthur JM. Urine biomarkers predict the cause of glomerular disease. Journal of the American Society of Nephrology. 2007;18(3):913-22. https://doi.org/10.1681/ASN.2006070767
21. Jain S, Rajput A, Kumar Y, Uppuluri N, Arvind A, Tatu U. Proteomic analysis of urinary protein markers for accurate prediction of diabetic kidney disorder. JAPi. 2005;53(513):20. Available from: https://web.archive.org/web/20190228203525id_/http://pdfs.semanticscholar.org/9808/4c06a1498d6fa14b506ae54746a2f54fdf53.pdf