Research Article

Urinary Pigment Epithelium-Derived Factor as a Marker of Diabetic Nephropathy in Egyptian Patients with type 2 diabetes mellitus

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Abstract
Introduction: Diabetic nephropathy (DN) is the most common cause of end-stage renal disease. Urinary pigment epithelium derived factor (PEDF) has also been shown to suppress the expression of fibrogenic, proinflammatory, and angiogenic factors, thus contributing to pathological changes in early DN. We aimed to study the the role of urinary PEDF as a biomarker for the detection of chronic kidney disease (CKD) progression in patients with type 2 diabetes mellitus (T2DM). **Methodology**: Sixty patients with T2DM were recruited in addition to twenty non diabetic healthy volunteers. Urinary PEDF using ELISA technique were performed to all subjects and correlations between it and different clinical parameters were examined. **Results**: our study showed statistically significant correlation between urinary PEDF level and duration of DM (P value < 0.001), HbA1C (P value < 0.001), serum creatinine (P value < 0.001), urinary A/C ratio (P value < 0.001), and stage of diabetic retinopathy by fundus examination (P value < 0.001). **Conclusion**: urinary PEDF is a good indicator of progression of diabetic nephropathy and microvascular damage (as a complication of diabetes) in general. It was also increased in case of poor diabetic control.
Keywords: Diabetic Nephropathy; Diabetic retinopathy; Albuminuria; Pigment Epithelium Derived Factor.

Introduction
Diabetic nephropathy (DN) is the most common cause of end-stage renal disease. It accounts for 54% of all renal failure patients requiring chronic dialysis[(1)]. Early detection can ensure timely intervention and improvement of treatment outcome. Thus far, microalbuminuria (MA) has been recognized as an early indicator of DN; however, the presence of albuminuria might not be always indicative of DN in individuals with type 2 diabetes mellitus (T2DM), as revealed by biopsy studies[(2)]. In fact, MA is a marker for endothelial dysfunction. MA may develop because of hypertension or/and insulin resistance[(3)], along with hyperglycemia, and the presence of MA in T2DM patients may also be indicative of cardiovascular disease.

Therefore, there is a need to identify new sensitive and specific markers for screening and assessing incipient DN and monitoring responses to therapy. Identification of novel biomarkers implicated in DN may enable early detection of patients at risk of clinical disease progression, especially before a significant reduction in glomerular filtration rate (GFR) or the development of microalbuminuria[(4)].

Pigment epithelium-derived factor, a member of the serine protease inhibitor (serpin) gene family, is a 50 Kilodaltons (KDa) glycoprotein first identified in the conditioned medium of human retinal pigment epithelial cells as a neurotrophic factor and a potent angiogenic inhibitor[(5)]. Cell-based and animal studies have suggested PEDF to be a local protective factor against diabetic microvascular damage[(6)]. Decreased PEDF protein and mRNA expression has been found in kidneys of diabetic mice[(7)]. PEDF has also been shown to suppress the expression of fibrogenic[(8)], proinflammatory, and angiogenic factors[(9)], thus contributing to pathological changes in early DN[(10)].

In the current study, we aimed to examine the role of urinary PEDF as a biomarker for the detection of chronic kidney disease (CKD) progression in patients with type 2 diabetes mellitus.

Patient and methods

Kasr Al- Ainy Hospital is a major hospital and a tertiary referral center serving patients from Cairo and also patients referred from all other governorates of Egypt. Sixty patients with type 2 diabetes mellitus were recruited from the outpatient endocrinology clinic, Nephrology clinic and Internal Medicine Departments during August 2016 to December 2018; in addition to twenty age and sex matched non diabetic healthy individuals were enrolled in this study as controls.

Diagnosis of diabetes based upon the WHO classification. At least 5 years from the diagnosis of type 2 diabetes was required to be included in our study.Exclusion criteria included Type 1 diabetes, End stage renal disease patients on regular hemodialysis, Cirrhotic patients, Child B and C, Patients on either angiotensin converting enzyme "ACE" inhibitors or angiotensin II receptor blocker ARB's, pregnant females, the Presence of infections and symptoms or signs of other systemic disease.

After discussing the procedure with each patient separately, a written consent was obtained. All our patients were subjected to full history, through clinical examination. The weight of each participant was measured using a digital scale while the participant was lightly clothed with bare feet. The height of each participant was measured while bare-feted, and the BMI was calculated as body weight in kgs divided by height square in meters (Kg/m2). The waist circumference was measured midway between rib margin and the iliac crest in a standing position by the same examiner. Blood pressure was measured using mercury sphygmomanometer. Two measurements were taken and averaged. Hypertension was diagnosed for any patient taking antihypertensive agents or his blood pressure >140⁄90 mmHg on two separate measurements on two different occasions. Fundus Examination: was performed to all participants using direct ophthalmoscopy, and results were classified according to International clinical diabetic retinopathy and diabetic macula edema disease severity scales[(11)]. Abdominal ultrasonography: was done using Siemens Acuson X300 device, with a convex 2D probe with frequency of 2.5 to 4.5 MHz after proper preparation of the patients, to assess size and echogenicity of both kidneys, and the presence of liver cirrhosis.

Laboratory investigations included: routine laboratory investigations including; Complete blood count (CBC), Fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c) and Serum levels of total proteins and albumin, renal function: Creatinine and BUN, estimated glomerular filtration rate (eGFR) according to MDRD (Modification of Diet in Renal Disease) equation, random urine sample for testing for albumin /creatinine ratio (ACR) repeated twice 3 months apart if the first sample was positive for micro-albuminuria, patients had been instructed to refrain from heavy exercise 24 hours before the test, Virology including hepatitis B virus surface antigen, hepatitis C virus antibody and human immunodeficiency virus antibody using ELISA technique and Urinary pigment epithelium derived factor (PEDF): by ELISA based on the Biotin double antibody sandwich technology (Add PEDF to the wells, which are pre-coated with PEDF monoclonal antibody, and then incubate, after that, add anti-PEDF antibodies labeled with Biotin to unite with Streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of human PEDF are positively correlated).

Statistical analysis

Pre-coded data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows. Data was then transferred to the Statistical Package of Social Science Software program (SPSS-25) to be statistically analyzed. Continuous variables are expressed as mean ± Standard deviation. Frequency and percentage were done for qualitative variables. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests[(12)]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5[(13)]. Correlations between quantitative variables were done using Spearman correlation coefficient[(14)]. A p value less than 0.05 was considered statistically significant, graphs were used to illustrate some information

Results

The present study included 80 subjects, 60 of which were diabetic patients (32 male and 28 female) and 20 were healthy control subjects (11 male and 9 female). Patients in our study were of average age (59.53 ± 10.52 years in the diabetic group and 47.30 ± 17.02 years in the control group). Diabetic group members had higher BMI (28.61 ± 4.89) compared to that of the control group (23.74 ± 4.34). Most of the diabetic group members were hypertensive (38 patients (63.3 %)). The average duration of diabetes in the diabetic group was 9.58 ± 5.54 years. Most of the diabetic patients were anemic. They had elevated serum creatinine (2.22 ± 1.77mg/dL), and albuminuria (about 1 gm in average). Diabetes in the diabetic group was not very well controlled (average HbA1C = 7.65 ± 1.24%). Laboratory results of studied groups discussed in **table (1)**. **Figure 1** showed the correlation between duration of DM and urinary A/C ratio. **Figure 2** showed the urinary PEDF level is significantly higher in diabetic group than control group.

Table 2 showed correlation between PEDF level and different parameters in the diabetic group. There was positive correlation between urinary PEDF level and Age (P value = 0.192), Duration of DM (P value < 0.001), HbA1C (P value < 0.001), Serum creatinine (P value < 0.001), Serum urea (P value < 0.001) in addition to Urinary A/C ratio (P value < 0.001) **Figure 3**. There was negative correlation between PEDF level and Serum hemoglobin (P value = 0.826), GFR (P value < 0.001), Serum potassium (P value = 0.839)in addition to Serum albumin (P value < 0.001).

Fundus examination results in the diabetic group were as follows Mild non-proliferative diabetic retinopathy (MNDR) was present in 38 patients (63.3 %), Severe non-proliferative diabetic retinopathy (SNDR) was present in 12 patients (20 %), Proliferative diabetic retinopathy (PDR) was present in 8 patients (13.3 %) while No apparent diabetic retinopathy (NDR) was present in 2 patients (3.3 %). **Figure 4** Showed the Correlation between A/C ratio and PEDF level with stage of diabetic retinopathy by fundus examination (using independent-samples Kruskal-Wallis test). The more progressive is the stage of diabetic retinopathy by fundus examination, the higher is the A/C ratio value. The more progressive is the stage of diabetic retinopathy by fundus examination, the higher is the PEDF level in urine.

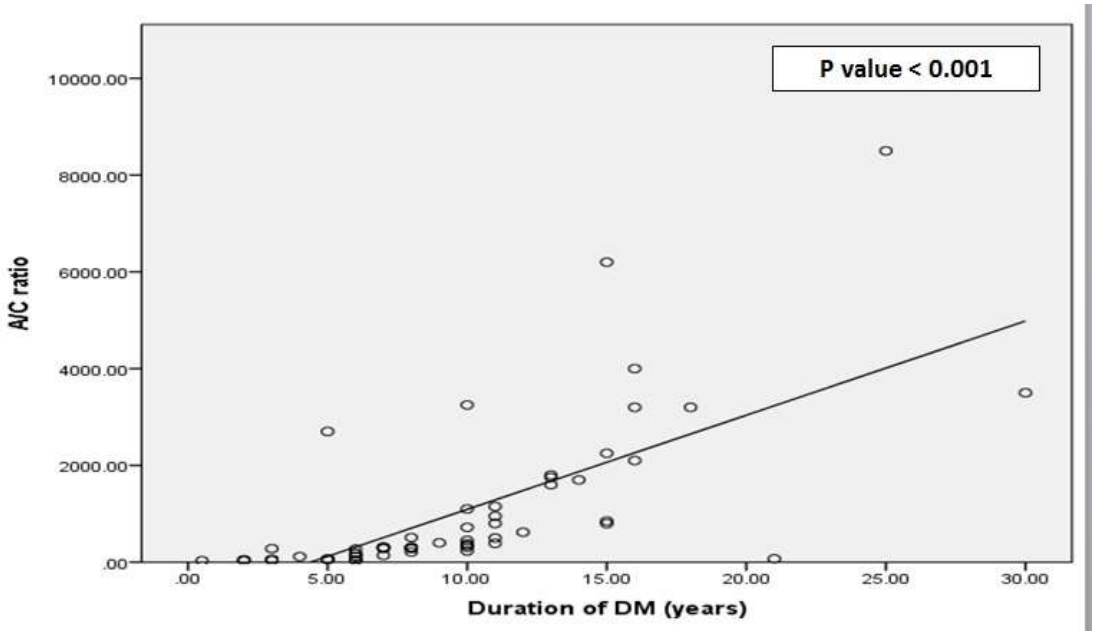

Figure 1: Correlation between duration of DM and urinary A/C ratio

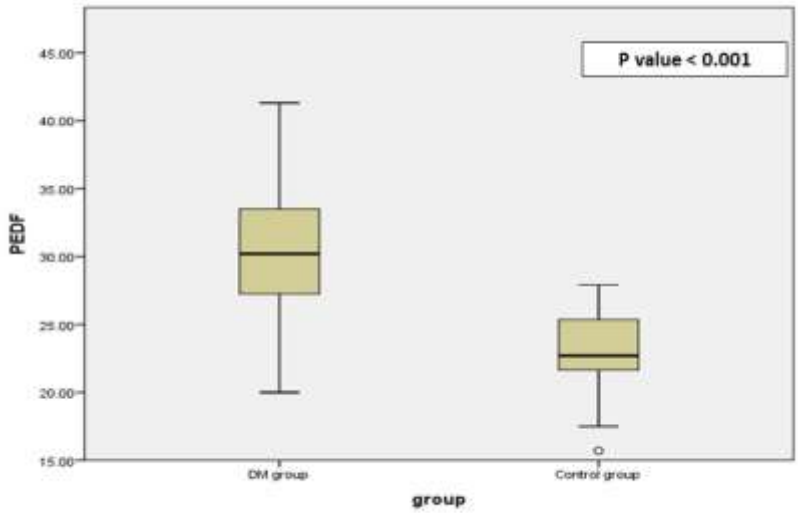

Figure 2: Comparison between urinary PEDF level in both diabetic and control groups

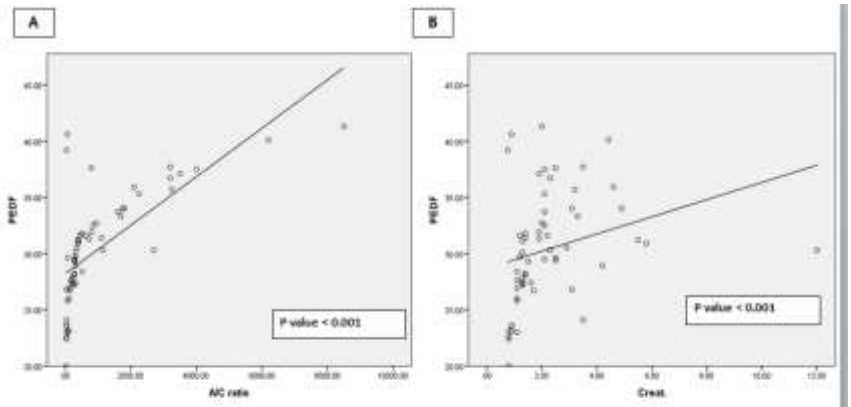

Figure 3: Correlation between PEDF level with urinary A/C ratio and serum creatinine
A: Correlation between PEDF level and urinary A/C ratio
B: Correlation between PEDF level and serum creatinine

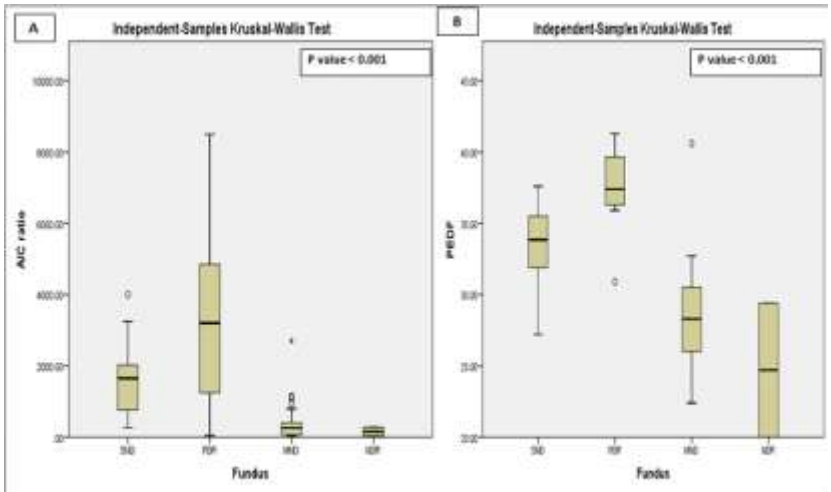

Figure 4: Correlation between PEDF level and urinary A/C ratio with stage of diabetic retinopathy by fundus examination (using independent-samples Kruskal-Wallis test)
A: Correlation between A/C ratio and stage of diabetic retinopathy by fundus examination
B: Correlation between PEDF level and stage of diabetic retinopathy by fundus examination

Table (1): Laboratory results of studied groups. K: potassium level**, eGFR:** estimated glomerular filtration rate**, A/C ratio:** albumin to creatinine ratio, **HbA_1C:** Glycated Hemoglobin

Parameter	Diabetic group Mean ± SD	Control group Mean ± SD	P value
Haemoglobin conc. (g/dL)	9.32 ± 1.86	12.27 ± 2.27	< 0.001
Creatinine (mg/dL)	2.22 ± 1.77	1.06 ± 0.32	< 0.001
Urea (mg/dL)	59.95 ± 32.98	38.50 ± 21.43	0.002
K (mEq/L)	4.39 ± 0.72	4.45 ± 0.61	0.789
eGFR (ml/min/1.73 m2)	43.79 ± 26.62	78.72 ± 28.11	< 0.001
Albumin (g/dL)	3.59 ± 0.67	4.19 ± 0.57	< 0.001
A/C ratio (mg/g)	1009.42 ± 1573.64	36.05 ± 77.12	< 0.001
HbA1C (%)	7.65 ± 1.24	5.55 ± 0.51	< 0.001

Table (2): Correlation between PEDF level and other parameters in the diabetic group. K: potassium level**, eGFR:** estimated glomerular filtration rate**, A/C ratio:** albumin to creatinine ratio, **HbA_1C:** Glycated Hemoglobin

Parameters in the diabetic group		PEDF
Age (years)	**Correlation Coefficient**	0.171
	P value	0.192
	Number	60
Duration of DM (years)	**Correlation Coefficient**	0.795
	P value	**< 0.001**
	Number	60
HbA1C (%)	**Correlation Coefficient**	0.946
	P value	**< 0.001**
	Number	60
Haemoglobin conc. (g/dL)	**Correlation Coefficient**	- 0.029
	P value	0.826
	Number	60
Creatinine (mg/dL)	**Correlation Coefficient**	0.521
	P value	**< 0.001**
	Number	60
Urea (mg/dL)	**Correlation Coefficient**	0.573
	P value	**< 0.001**
	Number	60
eGFR (ml/min/1.73 m^2)	**Correlation Coefficient**	- 0.511
	P value	**< 0.001**
	Number	60
K (mEq/L)	**Correlation Coefficient**	- 0.027
	P value	0.839
	Number	60
Albumin (g/dL)	**Correlation Coefficient**	- 0.538
	P value	**< 0.001**
	Number	60
A/C ratio (mg/g)	**Correlation Coefficient**	0.805
	P value	**< 0.001**
	Number	60

Discussion

Our study showed that PEDF level in urine is significantly higher in diabetic group than in control group (P value < 0.001). Chen et al in 2010 found the same results,[7]. Also our study showed that urinary PEDF level was positively correlated to duration of DM (r = 0.795, P value < 0.001), Serum creatinine (r = 0.521, P value < 0.001), A/C ratio (r = 0.805, P value < 0.001), and was negatively correlated to GFR (r = -0.511, P value < 0.001). All these results suggest that urinary PEDF is probably an important determinant of the pathogenesis of diabetic nephropathy.

Hui et al., in 2014 showed the same results but in correlation with serum PEDF instead of urinary PEDF,[8]. From this we conclude that both serum and urinary PEDF are equally effective as a marker for progression of diabetic nephropathy.

Several studies have suggested that albuminuria can be attributed with confidence to diabetic nephropathy, if diabetic retinopathy is present. In 2007, KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic kidney disease stated that in most patients with diabetes, chronic kidney disease should be attributable to diabetes if MA is present along with DR,[15]. Our study showed that the more progressive is the stage of diabetic retinopathy by fundus examination, the higher is the PEDF level in urine (P value < 0.001). This further proves that PEDF level is well correlated to progression of diabetic nephron-pathy, and that the presence of high PEDF level denotes the presence of microvascular damage (retinopathy, or nephropathy, e.t.c).

Also our study showed that urinary PEDF level is positively correlated to HbA_1C level (r = 0.946, P value < 0.001). So, we can conclude that PEDF level is also affected by the degree of diabetic control. The reason for increased urinary PEDF level in T2DM is unclear. It is speculated that there are several reasons behind the increased urinary concentrations of PEDF; (1) Hyperfiltration of circulating PEDF because of the increased permeability of the glomerular basement membrane. PEDF is a glycoprotein with a molecular weight of 50 kDa, which is close to albumin with a molecular weight of 65 kDa. (2) Increased production or secretion of PEDF by renal tissues in response to hyperglycemia. This hypothesis could be best confirmed by biopsy studies, but it is very difficult to perform such studies in human beings, but it was performed in mice in 2007[16].

Study Limitations:

First, the study used a cross-sectional design and included a relatively small sample size. Second, Lack of data about correlation between PEDF level and kidney disease progression in type 1 DM patients and Lack of information about whether PEDF level is affected by antiproteinuric drugs e.g. ACE inhibitors, or urinary tract infection.

Conclusion

Our study demonstrates that the urinary PEDF is a good marker for progression of diabetic nephropathy and microvascular damage -as a complication of diabetes- in general. It can be used instead of or in addition to A/C ratio for follow up of diabetic nephropathy progression.

Ethical Committee Approval

The local ethical committee of the Internal Medicine department, School of Medicine, Cairo University, approved this work.

Human and Animal Rights: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: "Informed consent was obtained from all individual participants included in the study".

Conflict of interest: The authors have declared that no conflict of interest exists.

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Reference

1. Locatelli F, Pozzoni P, Del Vecchio L. Renal replacement therapy in patients with diabetes and end-stage renal disease. J Am Soc Nephrol. 2004 Jan; 15 Suppl 1:S25-9.
2. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, et al., Nephropathy in diabetes. Diabetes Care. 2004 Jan;27 Suppl 1:S79-83.
3. Lin CY, Chen MF, Lin LY, Liau CS, Lee YT, Su TC. Insulin resistance is the major

determinant for microalbuminuria in severe hypertriglyceridemia: implication for high risk stratification. Intern Med. 2008; 47(12):1091-7.
4. Gerstein HC, Islam S, Anand S, Almahmeed W, Damasceno A, Dans A, et al., Dysglycaemia and the risk of acute myocardial infarction in multiple ethnic groups: an analysis of 15,780 patients from the INTERHEART study. Diabetologia. 2010 Dec; 53(12):2509-17.
5. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu HJ, Benedict W, et al., Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 2009; 285: 245-248.
6. Boehm BO, Lang G, Volpert O, Jehle PM, Kurkhaus A, Rosinger S, et al., Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retino-pathy. Diabetologia. 2003 Mar; 46(3):394-400.
7. Chen H, Zheng Z, Li R, Lu J, Bao Y, Ying X, et al., Urinary Pigment Epithelium-Derived Factor as a Marker of Diabetic Nephropathy. Am J Nephrol. 2010; 32(1): 47-56.
8. Hui E, Yeung CY, Woo YC, Woo YC, Fong CH, Chow WS, et al., Elevated Circulating Pigment Epithelium-Derived Factor Predicts the Progression of Diabetic Nephropathy in Patients With Type 2 Diabetes. J Clin Endocrinol Metab. 2014 Nov; 99(11):E2169-77.
9. Awad AS, Gao T, Gvritishvili A, You H, Liu Y, Cooper TK, et al., Protective role of small pigment epithelium-derived factor (PEDF) peptide in diabetic renal injury. Am J Physiol Renal Physiol. 2013 Sep 15; 305(6):F891-900.
10. Wang JJ, Zhang SX, Mott R, Chen Y, Knapp RR, Cao W, et al., Anti-inflammatory effects of pigment epithet-lium-derived factor in diabetic nephron-pathy. Am J Physiol Renal Physiol. 2008 May; 294(5):F1166-73.
11. Wilkinson CP, Ferris FL III, Klein RE, Lee PP, Agardh CD, Davis M, et al., Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003 Sep; 110 (9):1677-82.
12. Chan YH (a). Biostatistics 102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J. 2003; 44(8):391-396.
13. Chan YH (b). Biostatistics 103: Qualitative Data –Tests of Independence. Singapore Med J. 2003; 44(10): 498-503.
14. Chan YH (c). Biostatistics 104: Correlational Analysis. Singapore Med J. 2003; 44(12): 614-619.
15. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis 2007; 49: 12-154.
16. Sahin K, Onderci M, Tuzcu M, Ustundag B, Cikim G, Ozercan IH, et al., Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat. Metabolism. 2007 Sep; 56 (9):1233-40.