Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Assessment of serum Cystatin-C, Cortisol and Aldosterone hormone as markers for early detection of nephropathy among Iraqi patients with Type 2 Diabetes Mellitus

Refaa B AL-TEMIMI[1*], Amer Hasan ABDULLAH[1] Noor T TAHIR[2]

1 Department of Chemistry, College of Science, University of Al Mustansiriyah, Baghdad, Iraq
2 National Diabetes Center, Mustansiriyah University, Baghdad, Iraq
* Corresponding Author. E-mail: nephrology.1020@gmail.com (R.T.); Tel. +964 770 483 1020.

Received: 8 March 2024 / Revised: 24 March 2024 / Accepted: 26 March 2024

ABSTRACT: Biomarkers which identify Diabetic nephropathy (DN) patients could allow early, more efficient intervention and management, decreasing patient morbidity and mortality. This study aimed to estimate the predictive value of cystatin-c protein, and the hormones cortisol and aldosterone in detecting DN in a study including 60 patients with DN under hemodialysis (HD) and other 60 patients with type 2 diabetes mellitus (T2DM) who were recruited from AL-Yarmouk, AL-Kindy and AL- Karama Teaching Hospitals' dialysis center. Serum levels of cystatin-c, cortisol, and aldosterone were measured for both groups. Serum cystatin-c protein, cortisol and aldosterone hormones levels were significantly higher in patients with DN under HD when it compared with T2DM patients. Receiver operating characteristic (ROC) curve was utilized to test the cut off values of these biomarkers to predict DN results revealed that serum cystatin-c, cortisol and aldosterone levels were significantly higher in patients with DN under HD when it compared with T2DM patients. ROC analysis revealed that the cystatin-C level had the best predictive value in detecting of DN compared with serum cortisol and aldosterone which indicate that cystatin-C could be used to predict DN development and progression in patients with T2DM

KEYWORDS: Diabetes mellitus; Diabetic nephropathy; Cystatin-c; Cortisol; Aldosterone.

1. INTRODUCTION

Diabetes mellitus (DM) is considered as one of the most challenging health issues in the current era. Type2-diabetes mellitus (T2DM) represent about (85% - 95%) of total diabetic issues in developed nations and countries and accounts for biggest percentage in leading countries [1]. Individuals diagnosed with T2DM are inherently susceptible to tissue damage in organs that are supplied by an end arterial system as a result of microangiopathy. These microvascular complications Include diabetic nephropathy (DN), diabetic retinopathy, and diabetic neuropathy. The progression of DN begins with the emergence of abnormally low amounts of albumin in urine (≥30mg - 299 mg/day), known as microalbuminuria. This then advances to the stage of macro albuminuria (≥300mg/day), and ultimately leads to End-Stage Renal Disease (ESRD). Micro-albuminuria may still under progression to macro-albuminuria in (10-15 years) [2,3]. Epidemiological researches have clarified that the mortality ratio of DM patients with DN is 30 time higher than the patients without kidney disease complications; so, earlier characterization and medication of DN is essential for developing the quality of life (QOL) of DM patients [4].

Cystatin-C (Cys-C), is a non-glycosylated with low molecular weight protein (13.4 k Da) belonging to the cystatin superfamily of cysteine protease inhibitors [5]. It's produced at a constant rate by all nucleated cells and is expressed in relatively high concentrations in serum, saliva, seminal, and cerebrospinal fluids [6]. Unlike creatinine, it does not undergo renal secretion from tubule and is not influenced by extra-kidney factors like age, race, gender, diet, and muscle mass, so Cys-C levels are a more sensitive indicator of kidney function and estimate the GFR than serum creatinine levels [7,8].

Cortisol (hydrocortisone), a hormone belongs to the glucocorticoid hormones, is produced by the adrenal glands. It assists cells to make energy [9]. When a body experiences stress, even it was physical, mental, or emotional, cortisol reproduction by the adrenals is increased in order to conserve the body. Patients with T2DM and chronic kidney disease (CKD) Individuals encounter numerous stressful circumstances in their daily lives. The majority of them exhibit several co-morbidities and have diverse

How to cite this article: Al-Temimi RB, Abdullah AH, Tahir NT. Assessment of Serum Cystatin-C, Cortisol and Aldosterone Hormone as Markers for Early Detection of Nephropathy among Iraqi Patients with Type 2 Diabetes Mellitus. J Res Pharm. 2025; 29(1): 469-475.

https://dx.doi.org/10.29228/jrp.2022.00
ISSN: 2630-6344

social, economic, and medical challenges. Since T2DM and chronic kidney disease (CKD) patients are living in stressful conditions; it may be feasible that cortisol concentrations could be connected with these circumstances [10].

The renin-angiotensin-aldosterone system (RAAS) is essential causes in the pathogenesis of DN. The activation of the local RAAS leads to a rise in angiotensin II, as well as the activity of adrenocorticotropic hormone (ACTH) and potassium. These three variables are primarily responsible for regulating aldosterone secretion [4]. Aldosterone stimulates the reabsorption of sodium through the mineralocorticoid receptor on cells lining the renal tubules. This leads to an increase in fluid volume and blood pressure, which is strongly associated with proteinuria and the progression of kidney injury [11,12]. The purpose of the present study is to evaluate the diagnostic value of Cys-c protein and hormones cortisol and aldosterone for discriminating between T2DM and DN.

2. RESULTS

2.1. Demographic baseline of the study population

As indicated in Table 1, no significantly differences were obtained in age among patients in DN under hemodialysis (HD) and T2DM patients. Body mass index (BMI) was significantly higher in T2DM patients (32.95±3.08) compared with DN under HD patients (27.18±5.58). The duration of DM in patients with DN under HD was significantly higher than patients with T2DM without DN (7.0years vs. 3.5 years).

Table 1. Demographic profile of the study sample

Variable	DN under HD (n=60)	T2DM (n=60)	p-value
Age, years			
Mean ±SD	46.37±12.07	44.6±5.41	**0.762**
Range	30-72	30-70	
BMI, Kg/m²			
Mean ±SD	27.18±5.58	32.95±3.08	**<0.001**
Range	16.16-46.81	24.72-36.73	
Duration of DM, M			
Mean ±SD	114±3.85	4.11±3.12	**<0.001**
Median	7.0	3.5	
Range	1.0-15.0	0.1-12.0	

2.2. Determination of biochemical tests

Table 2 demonstrated that mean value of HbA1c in Patients with T2DM (8.31±1.34%) was higher than patients with DN under HD (6.6±1.73%). In the same table, the results revealed that there were non-significant difference in levels of FBG between patients with DN under HD and T2DM patients (181.32±44.2 mg/dl and 181.68±39.41 mg/dl, respectively). The mean value of blood urea and creatinine in the patients with DN under HD were (153.83±47.04 mg/dl and 8.08±2.54 mg/dl, respectively) compared 35.80±7.55 mg/dl and 1.48±2.10 mg/dl, respectively in T2DM with highly significant differences. While, GFR was significantly lower in patients DN under HD (7.6±2.90 ml/min/1.73m²) than those with T2DM (96.12±12.30 ml/min/1.73m²).

Table 2. Mean ±SD values of biochemical tests

Variable	DN under HD (n=60)	T2DM (n=60)	p-value
FBS, mg/dl Mean ±SD	181.32±44.2	181.68±39.41	0.962
Range	133-320	130-248	
HbA1c, % Mean ±SD	6.6±1.73	8.31±1.34	**<0.001**
Range	4.3-11.5	6.3-11.3	
Urea, mg/dl Mean ±SD			
Range	153.83±47.04	35.80±7.55	**<0.001**
	28.7-270.41	20.0-64.0	
Creatinine, mg/dl Mean ±SD	8.08±2.54	1.48±2.10	**<0.001**
Range	4.04-13.48	0.50-9.0	
GFR, ml/min/1.73m² Mean ±SD			
Range	7.6±2.90	96.12±12.30	**<0.001**
	4.0-14.0	64.0-121.0	

2.3. Determination of Cystatin-C, Cortisol and Aldosterone

As depicted in table 3, the results showed that the median value of the Cys-C was significantly higher in DN under HD patients (25.75 ng/ml) compare with T2DM patients (8.3 ng/ml). The median serum level of cortisol in patients with T2DM was significantly lower than that of patients with DN under HD (median= 10.51 pg/ml, 14.43 pg/ml, respectively). Also, aldosterone level was significantly higher in patients with DN under HD (median= 56.73 ng/ml, range= 17.81-179.4 ng/ml) than patients with T2DM (median= 24.48 ng/ml, 11.09-59.53 ng/ml).

Table 3. Cys-C and hormonal profile among study population.

Variable	DN under HD(n=60)	T2DM (n=60)	p-value
Cys-c, ng/ml			**<0.001**
Mean±SD	30±34.71	8.76±1.34	
Median	25.75	8.3	
Range	10.0-292	6.50-12.2	
Cortisol, mcg/dl			
Mean±SD	15.14±7.35	11.88±5.75	**0.008**
Median	14.43	10.51	
Range	2.15-40.86	3.36-38.19	
Aldosterone, pg/ml			
Mean±SD	58.47±29.16	26.51±11.46	**<0.001**
Median	56.73	24.48	
Range	17.81-179.4	11.09-59.53	

2.4. ROC analysis of cystatin-c, cortisol and aldosterone

The Receiver operating characteristic (ROC) curve analyses of cystatin-c, cortisol and aldosterone in anticipating DN in patients with T2DM are depicted in Figure 1 and Table 4.

The area under the curve to diagnose established diabetic nephropathy in type 2 DM using Cys-C was 0.997, 95%CI=0.991-1.0, p< 0.001 with specificity and sensitivity 100% and 98%, respectively at cut-off value of = 14.65 ng/ml. For cortisol, the AUC was 0.647, 95%CI= 0.547-0.748, p= 0.001. The sensitivity and specificity at cut off value of cortisol = 12.75 mcg/ml were 62% and 61%, respectively. For aldosterone, the AUC was 0.893, 95%CI=0.833-0.953, p< 0.001. The sensitivity and specificity at cut off value of aldosterone = 34.34 pg/ml were 87% and 88%, respectively

Table 4. ROC analysis results for Cys-C, Cortisol and aldosterone

Marker	AUC	95%CI	Specificity	Sensitivity	Cut-Off value	P-Value
Cys-C	0.997	0.991-1.0	100	98	14.65	< 0.001
Cortisol	0.647	0.547-0.748	61	62	12.75	0.001
Aldosterone	0.893	0.833-0.953	88	87	34.34	p< 0.001

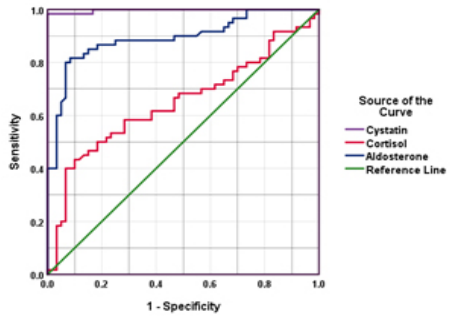

Figure 1. ROC for cystatin-c, cortisol and aldosterone in the context of discrimination between T2DM and DN.

3. DISCUSSION

Many individuals with T2DM undergoes a phase of pre-diabetes and may encounter renal impairment. Indeed (20-40%) of people with diabetic will Advancement to more grave renal disorders e.g. DN. So, detection of renal dysfunction at earlier stage is substantial as early Interference can slow down the loss of kidney role, that Enhances survival and life quality [2,13]. It is obvious from the results of the current study that the mean age of DN patients under HD was not- significantly differ from that of non-DN T2DM patients. These results are compatible with previous studies which reported that a non-significant difference

in age was observed between DN patients under HD and non-DN T2DM [14, 15]. It has been suggested that the prevalence of CKD is increasing in the elderly can be due to an increase in age related risk factors for progression to the CKD such as diabetes, hypertension and cardiovascular disease [16]. Furthermore, BMI were significantly higher in non-DN T2DM patients compared with DN patients under hemodialysis in agreement with results reported previously [17, 18]. Body mass index is widely utilized as a risk factor in the development or prevalence of several health problems. The present results reported previously which stated that individuals with BMI ≥30 kg/m^2 are at substantially heightened lifetime risk of diabetes, having even moderately elevated BMI is associated with increased risk of developing diabetes mellitus complications [19]. The BMI was lower in patients with hemodialysis as this condition represents a chronic depletion condition that affects or increases metabolic rate and causes severe weight lost in addition to frequent dialysis and strict dietary habits that reduces protein intake [20].

Results obtained demonstrated that mean FBG did not differ significantly between patients DN under HD and non-DN T2DM patients .Whereas HbA1c in patients with non-DN T2DM patients was higher significantly than that of DN patients under HD in agreement with results demonstrated previously [14, 17, 18].

The explanation of these findings might be due to that during hemodialysis, the uremic environment, blood loss during treatments, and frequent phlebotomy all contribute to decreased RBC lifespan. So shortened RBC survival and red cell transfusions are likely to lower the HbA1c.Also, lower HbA1c values were associated with lower hemoglobin concentration and higher doses of erythropoietin [3, 4]. The glycation rate of just-produced young erythrocytes is reported to be lower than that of old cells. Therefore, it seems that the decrease of HbA1c levels relative to FBG in HD with diabetes patients and are treated with erythropoietin might be due to the increasing proportion of young erythrocytes over old erythrocytes in peripheral blood of those patients [21, 22].

Serial determinations of serum Cys-C accurately diagnosed trends in kidney function in people with normal or high glomerular filtration rate (GFR) and offer a method for investigating early loss in renal function in individuals with diabetes [23]. The findings of our study indicated that a significant proportion of individuals with diabetes experienced an elevation in their serum levels of Cys-C. Consistent with previous research, our findings indicate that Cys-C may have a function in the progression of DN in individuals with diabetes. [4]. Many previous studies have provided evidences that serum Cys-C is a better sensitive biomarker for diagnosis of earlier changes for in glomerular filtration in T2DM patients than and creatinine, because of its independence of age, BMI and gender. Also, other study was Performed by Gupta et al. clarify that the Cys-C concentrations were elevated even in the patients that clinical albuminuria were not started yet, Therefore, it serves as an earlier indicator than microalbuminuria in identifying nephropathy [24,25]. The limited capacity of creatinine to identify early deterioration in glomerular filtration rate (GFR) is attributed to the fact that serum creatinine level (SCr) only starts to elevate above the normal value when approximately 50% of renal function is losted, suggesting that GFR can change before SCr going to be abnormal [13].

Studies that conducted on rats observe that renal clearance of Cys-C is 94% of the Often-utilized GFR parameters Cr EDTA and that Cys-C thus is actually filtered in the glomeruli freely. Under normal circumstances, the filter of glomerular is clears compounds with MWT up to 58,000 Dalton while in ESRD these solutes are retained i.e. Cys-C leading to its increased serum concentrations [26]. This explains the high level of Cys-C in DN under HD compared with T2DM in the present study.

Recently, Sun, S. and Wang, Y. investigated whether the levels of blood cortisol are linked to the occurrence of microvascular complications in patients with (T2DM). The level of cortisol had a favorable correlation with the degree of microalbuminuria, as well as with the severity of diabetic retinopathy and neuropathy [27]. The authors speculated that raised excretion of cortisol may be contributed to the advancement of diabetes and its emergence of micro-albuminuria. Zhang, X., et al ,2020 evaluate the relationship among serum cortisol concentrations and the existence of micro albuminuria in patients with T2DM and pre-diabetes. Serum levels of cortisol were significantly higher in microabuminuria group than in normoalbuminuria group. After amendment for multiple factors, the correlation among cortisol concentration and microalbuminuria remained consistent and significant. The authors concluded that increased values of cortisol, even when it in the normal value, may be connected to the Progress of micro albuminuria [28]. The explanation for such an association was that the high level of cortisol may lead to the raise of visceral fat amount, that in turn leads to the raise of plasma free fatty acid (FFA) vales. An excess amount of FFA would attach to serum albumin, leading to an increase in the excretion of albumin into the urine. Elevated levels of FFA can potentially trigger the activation of the protein kinase-C (PKC) pathway in

kidney vascular endothelial cells, that has been demonstrated to have a significant impact on the development of DN [29].

Aldosterone is a key risk factor promoting inflammation and fibrosis leading to renal complications. Plasma aldosterone concentration is elevated in patients with diabetes mellitus [30]. Denecke B., et al,2003 Exhibited that the plasma aldosterone value is inversely related with insulin sensitivity [31]. Furthermore, there is enormous experimental realities that aldosterone contributes to the Progress of nephron-sclerosis and kidney fibrosis in exemplars of diabetes and hypertension [23].

Previous data showed that elevated levels of circulating aldosterone are associated with a malfunction in the insulin-signaling pathway and impaired functioning of the endothelium, resulting in insulin resistance and injury to the kidneys [33]. Various mechanisms have been suggested to explain the development of insulin resistance caused by aldosterone, including altered function of pancreatic β-cells, reduced insulin sensitivity in target organs, and the promotion of deleterious adipocytokine production in adipose tissue [34].

Finally, the present study found as shown in figure 1 that Cys-C had sensitivity and specificity higher than cortisol and aldosterone hormone; therefore, Cys-C could be a good and an earlier marker for the diagnosis of nephropathy in patients withT2DM.

4. CONCLUSION

The results obtained from this research work supposed that the evaluation of Cys-C protein in serum of patients having T2DM is a more efficient method than cortisol and aldosterone hormones for early detection of kidney dysfunctionin of patients with T2DM. These findings neeed further investigation to elucidate the exact role of Cys-C protein as a diagnostic marker for DN.

5. MATERIALS AND METHODS

5.1. The Study Population

This study included 120 patients with T2DM, ages range 30-72 years. They were recruited from AL-Karama Teaching Hospital, AL- Yarmouk Teaching Hospital, and AL-Kindy Teaching Hospital in Baghdad / Iraq for the period from January 2022 to June 2022. The participants were divided into two primary categories: 60 patients without DN and 60 patients with DN undergoing hemodialysis treatment 3 times a week for 3 hours/ session.

Exclusion criteria included the individuals with Type I diabetes mellitus, hypo or hyperthyroidism, clinically confirmed depression, and has undergone renal transplantation, other patients with renal disease such as systemic lupus erythematosus. A comprehensive questionnaire comprising specific demographic information such as gender, age, and duration of diabetes, hemodialysis (HD) duration, each participant's treatment type (whether dietary, oral, insulin, or combined therapy) and parents' history of diabetes were collected. Anthropometric measurements, including height and body weight, and body mass index (BMI) were evaluated in all patients subjected to the study according to standard protocols.

5.2. Sample collection

About 10 mL of venous blood were drawn from patients with T2DM and DN under HD (before taking the dose of heparin) by venipuncture, and splitted to two aliquots; 5 ml in ethylene diamine tetra acetic acid (EDTA) (1.5 mg/mL) for determination of HbA1c%., while the second aliquot were moved into plain tube without anticoagulant, permitted to clump for thirty minutes, centrifuged for 15 minutes at 3000 xg. Separated sera were frozen in tightly sealed Eppendorf tubes and stored at -20°C for biochemical tests.

5.3. Biochemical Tests

Ready commercial kits (Elecsys / Roche Diagnostics GmbH, Germany) were used for measuring serum level of cortisol hormone. The cobas e 411 instrument is a full automated analyzer which utilized a patented Electro ChemiLuminescence (ECL) technology of immunoassay analysis for cortisol hormone. A ready commercial kit (Melsin/China) was utilized to quantify serum concentration of Cys-C and aldosterone hormone using enzyme linked immune-sorbent assay. Absorbance was taken at 450 nm and a standard curve was calculated from the standard dilution of Cys-C and aldosterone hormone.

5.4. Statistical analysis

The results of the present study have been analyzed by using SPSS version 25.0 (SPSS, Chicago). Continuous results were presented as mean and SD (or median and range as required), and analyzed by the

use of Student t-test. ROC analysis was utilized to estimate the predictive level of cystatin-C, cortisol and aldosterone hormone in predicting DN among patients with T2DM. A p-value of <0.05 was regarded to be significant [35,36].

Acknowledgements: The authors deeply thank the department of chemistry/college of sciences/ mustansiriah university / Baghdad-Iraq for their help in providing the scientific laboratories to make this work done. A special thanks to all the participants in this study.

Author contributions: Concept – A.A., N.T.; Design – R.T., A.A., N.T.; Supervision – A.A., N.T.; Resources – R.T., A.A.; Materials – R.T.; Data Collection and/or Processing – R.T.; Analysis and/or Interpretation – R.T., A.A.; Literature Search – R.T., N.T.; Writing – R.T.; Critical Reviews A.A., N.T.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Inci A, Sari F, Coban M, Olmaz R, Dolu S, Sarıkaya M, Yılmaz N. Soluble Klotho and fibroblast growth factor 23 levels in diabetic nephropathy with different stages of albuminuria. J Investig Med. 2016;64(6):1128-1133. https://doi.org/10.1136/jim-2016-000142

[2] Harkin C, Cobice D, Brockbank S, Bolton S, Johnston F, Strzelecka A, Watt J, Kurth MJ, Lamont JV, Fitzgerald P, Moore T, Ruddock MW. Biomarkers for detecting kidney dysfunction in Type-2 diabetics and diabetic nephropathy subjects: A case-control study to ıdentify potential biomarkers of DN to stratify risk of progression in T2D Patients. Front Endocrinol (Lausanne). 2022;13:887237. https://doi.org/10.3389/fendo.2022.887237

[3] Shelke SN, Tele JS. Cystatin C based eGFR - for early detection of diabetic kidney disease. Int J Res Med Sci. 2019;7(9):3402–3406. https://doi.org/10.18203/2320-6012.ijrms20193921

[4] Salih BH, Ali SH, Al-Lehibi KI. Serum aldosterone levels in patients with diabetic nephropathy in relation to vascular calcification. Iraqi J Pharm Sci. 2019;28(1):53–63. https://doi.org/10.31351/vol28iss1pp53-63

[5] Javanmardi M, Azadi NA, Amini S, Abdi M. Diagnostic value of cystatin C for diagnosis of early renal damages in type 2 diabetic mellitus patients: The first experience in Iran. J Res Med Sci. 2015;20(6):571-576. https://doi.org/10.4103%2F1735-1995.165960

[6] Lujambio I, Sottolano M, Luzardo L, Robaina S, Krul N, Thijs L, Carusso F, da Rosa A, Ríos AC, Olascoaga A, Garau M, Gadola L, Noboa O, Staessen JA, Boggia J. Estimation of glomerular filtration rate based on serum Cystatin C versus creatinine in a Uruguayan Population. Int J Nephrol. 2014;2014:837106. https://doi.org/10.1155/2014/837106

[7] Zhang X, Liu X, Su G, Li M, Liu J, Wang C, Xu D. pH-dependent and dynamic interactions of cystatin C with heparan sulfate. Commun Biol. 2021;4(1):198. https://doi.org/10.1038/s42003-021-01737-7

[8] Stankute I, Radzeviciene L, Monstaviciene A, Dobrovolskiene R, Danyte E, Verkauskiene R. Serum Cystatin C as a biomarker for early diabetic kidney disease and dyslipidemia in young type 1 Diabetes patients. Medicina (Kaunas). 2022;58(2):218. https://doi.org/10.3390/medicina58020218

[9] Lee DY, Kim E, Choi MH. Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. BMB Rep. 2015;48(4):209-216. https://doi.org/10.5483/bmbrep.2015.48.4.275

[10] Drechsler C, Ritz E, Tomaschitz A, Pilz S, Schönfeld S, Blouin K, Bidlingmaier M, Hammer F, Krane V, März W, Allolio B, Fassnacht M, Wanner C. Aldosterone and cortisol affect the risk of sudden cardiac death in haemodialysis patients. Eur Heart J. 2013;34(8):578-587. https://doi.org/10.1093%2Feurheartj%2Fehs361

[11] Liu K, Zou H, Fan H, Hu H, Cheng Y, Liu J, Wu X, Chen B, You Z. The role of aldosterone in the pathogenesis of diabetic retinopathy. Front Endocrinol (Lausanne). 2023;14:1163787. https://doi.org/10.3389%2Ffendo.2023.1163787

[12] Higa M, Ichijo T, Hirose T. Aldosterone-to-renin ratio ıs associated with diabetic nephropathy in Type 2 Diabetic Patients: A single-center retrospective study. Med Sci Monit. 2022;28:e935615. https://doi.org/10.12659%2FMSM.935615

[13] Sangeeta S, Ambekar J, Sudhakar T, Shannawaz M, Dongre N. Assessment of eGFR, using Cystatin-C and creatinine based equations for the early detection of renal ınjury in diabetic and non diabetic patients.J Clin Diagn Res. 2018; 12(9):BC30-BC33. http://dx.doi.org/10.7860/JCDR/2018/36698.12069.

[14] Hayashi A, Takano K, Masaki T, Yoshino S, Ogawa A, Shichiri M. Distinct biomarker roles for HbA1c and glycated albumin in patients with type 2 diabetes on hemodialysis. J Diabetes Complications. 2016;30(8):1494-1499. https://doi.org/10.1016/j.jdiacomp.2016.08.015

[15] Fahad SM, Rashied RM, Jaffal WN. Effect of hemodialysis on some biochemical parameters in diabetic nephropathy patients. Indian J Forensic Med Toxicol. 2021;15(1):1499-1504. http://dx.doi.org/10.37506/ijfmt.v15i1.13624

[16] Alzamanan MD, Rayshan A, Salem A, Alyami A. Risk factors of chronic renal failure in adult patients at King Khalid Hospital, Najran City, Saudi Arabia. Egypt J Hosp Med. 2018;70(1):88-91. http://dx.doi.org/10.12816/0042967

[17] Sany D, Elshahawy Y, Anwar W. Glycated albumin versus glycated hemoglobin as glycemic indicator in hemodialysis patients with diabetes mellitus: variables that influence. Saudi J Kidney Dis Transpl. 2013;24(2):260-273.
[18] Sørensen VR, Mathiesen ER, Watt T, Bjorner JB, Andersen MV, Feldt-Rasmussen B. Diabetic patients treated with dialysis: complications and quality of life. Diabetologia. 2007;50(11):2254-2262. https://doi.org/10.1007/s00125-007-0810-1
[19] Palmer MK, Toth PP. Trends in lipids, obesity, metabolic syndrome, and Diabetes Mellitus in the United States: An NHANES Analysis (2003-2004 to 2013-2014). Obesity (Silver Spring). 2019;27(2):309-314. https://doi.org/10.1002/oby.22370
[20] Kojima D, Washida N, Uchiyama K, Hama EY, Nagasaka T, Kusahana E, Nakayama T, Nagashima K, Sato Y, Morimoto K, Kanda T, Itoh H. The body mass index change is associated with death or hemodialysis transfer in Japanese patients initiating peritoneal dialysis. Ren Fail. 2023;45(1):2163904. https://doi.org/10.1080%2F0886022X.2022.2163904
[21] Wang S, Hou X, Liu Y, Lu H, Wei L, Bao Y, Jia W. Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus. Cardiovasc Diabetol. 2013;12:146. https://doi.org/10.1186/1475-2840-12-146
[22] Jiang Z, Wang J, Zhao P, Zhang L, Guo Y. HbA1c: High in acute cerebral infarction and low in brain trauma. Prog Mol Biol Transl Sci. 2019;162:293-306. https://doi.org/10.1016/bs.pmbts.2019.01.008
[23] Al-Nasrawii MS, Jasim, BS, Hassan, SH. A comparatıve assessment of serum creatınıne and cystatın c as a sıgnıfıcance of nephropathy ın dıabetıc patıents. Malays J Public Health Med. 2020;20(3):189–194. https://doi.org/10.37268/mjphm/vol.20/no.3/art.527
[24] Gupta K, Nayyar SB, Sachdeva JK, Kumar P. Cystatin C in the early diagnosis of diabetic nephropathy and its correlation with albuminuria. Int J Adv Med. 2017;4(1):56. http://dx.doi.org/10.18203/2349-3933.ijam20170020
[25] Huh JH, Choi E, Lim JS, Lee MY, Chung CH, Shin JY. Serum cystatin C levels are associated with asymptomatic peripheral arterial disease in type 2 diabetes mellitus patients without overt nephropathy. Diabetes Res Clin Pract. 2015;108(2):258-264. https://doi.org/10.1016/j.diabres.2015.02.006
[26] Kutum DT, Mattack N, Devi R, Patgiri D. A study of serum Cystatin C in chronic kidney disease patients undergoing hemodialysis. J Med Sci Clin Res. 2016;04(10); 13187-13195. https://dx.doi.org/10.18535/jmscr/v4i10.46
[27] Sun S, Wang Y. Relationship between cortisol and diabetic microvascular complications: A retrospective study. Eur J Med Res. 2023;28(1):391. https://doi.org/10.1186%2Fs40001-023-01325-x
[28] Zhang X, Deng X, Zhou J, Qiu K, Deng M, Lin Z, Mosha SS, Li W. The association of serum cortisol level with microalbuminuria in patients with type 2 diabetes and prediabetes. Int J Med Sci. 2020;17(18):2998–3004. https://doi.org/10.7150%2Fijms.48742
[29] Dubois D, Chanson P, Timsit J, Chauveau D, Nochy D, Guillausseau PJ, Lubetzki J. Remission of proteinuria following correction of hyperlipidemia in NIDDM patients with nondiabetic glomerulopathy. Diabetes Care. 1994;17(8):906–908. https://doi.org/10.2337/diacare.17.8.906
[30] Frimodt-Møller M, Persson F, Rossing P. Mitigating risk of aldosterone in diabetic kidney disease. Curr Opin Nephrol Hypertens. 2020;29(1):145-151. https://doi.org/10.1097/mnh.0000000000000557
[31] Denecke B, Gräber S, Schäfer C, Heiss A, Wöltje M, Jahnen-Dechent W. Tissue distribution and activity testing suggest a similar but not identical function of fetuin-B and fetuin-A. Biochem J. 2003;376(Pt 1):135-145. https://doi.org/10.1042/bj20030676
[32] Náray-Fejes-Tóth A, Fejes-Tóth G. The SGK, an aldosterone-induced gene in mineralocorticoid target cells, regulates the epithelial sodium channel. Kidney Int. 2000;57(4):1290-1294. https://doi.org/10.1046/j.1523-1755.2000.00964.x
[33] Otsuka H, Abe M, Kobayashi H. The effect of aldosterone on cardiorenal and metabolic systems. Int J Mol Sci. 2023;24(6):5370. https://doi.org/10.3390%2Fijms24065370
[34] Luther JM. Effects of aldosterone on insulin sensitivity and secretion. Steroids. 2014;91:54-60. https://doi.org/10.1016%2Fj.steroids.2014.08.016.
[35] Abdulamir HA, Aldafaay AAA, Al-Shammari AH. The role of liver function tests in monitoring the effect of enzyme replacement therapy in children with Gaucher Disease. Res J Pharm Technol. 2022; 15(8):3490-3496. https://doi.org/10.52711/0974-360X.2022.00585.
[36] Al-Shammari AH, Khudhur A, Abdulamir HA. Effect of intralesional levofloxacin in the treatment of cutaneous leishmaniasis: Intralesional levofloxacin for cutaneous leishmaniasis treatment. J Popul Ther Clin Pharmacol. 2023;30(1):e92–e100. https://doi.org/10.47750/jptcp.2023.1028.