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Original Article

Serum Cystatin C Level as Predictor for Chronic Kidney Disease Stages in Nephropathy Patients with Type 2 Diabetes

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Abstract: Diabetes is a non-communicable disease that has increased rapidly in Vietnam within the last ten years. This disease affects the health of patients with many dangerous complications. In particular, kidney complications are due to microvascular complications within a high proportion of patients. Cystatin C seems to be a significant marker protein for the early diagnosis of kidney complications. Our study was conducted on 79 patients to assess the serum cystatin C level and its relationship with several factors. Results showed that cystatin C levels increased in patients with kidney damage, which was related to creatinine levels and estimated glomerular filtration rate using serum creatinine levels ($eGFR_{Cre}$). Our study showed that cystatin C is a valuable marker in diagnosing kidney damage earlier than creatinine.

*Keywords***:** Cystatin C, diabetic nephropathy, chronic kidney disease.

1. Introduction

Diabetes mellitus (DM) is a disease recorded with a high incidence globally, of 425 million [1]. It also inflicts a considerable socioeconomic burden worldwide estimated at $1.32 trillion in 2015 [2], affecting the lives of patients, their families, and the whole of society. 90% of cases with diabetes are DM type 2 (T2DM) [3]. Unfortunately, the disease is usually diagnosed when the patients have developed many complications of both the micro and macro vasculature [4]. In particular, it is counted as a high incidence with 20-40% of cases having renal complications, which is the leading cause of end-stage kidney disease [4]. These severe

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symptoms also increase the risk of other life-changing and life-threatening conditions such as coronary artery disease and stroke [5].

Glomerular filtration rate (GFR) less than 60 mL/minute/1.73 m^2 is an indicator used to determine impaired renal function. Previously, GFR was often estimated based on serum creatinine concentration, but this index is influenced by other patient factors such as age, gender, and in particular, muscle mass [6]. Therefore, many other indices have been studied to contribute to the assessment of renal function. In particular, cystatin C is highly recommended by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) to evaluate and classify chronic kidney disease [7].

In human blood, cystatin C is a low molecular weight (13,359 kDa) endogenous cysteine proteinase inhibitor, produced at a constant rate by nucleated cells. It is freely filtered by the glomerulus and reabsorbed in the proximal tubular cells with no further excretion [8, 9]. Therefore, serum cystatin C levels are valuable for the evaluation of GFR [8]. Many studies have shown that blood cystatin C concentration is not affected by age, gender, diet, nutritional or inflammation status [9, 10]. A study by Cheuiche et al., (2019) concluded that the formula to calculate GFR based on blood cystatin C concentration gives reliable results in diabetic patients [11]. These studies show many advantages of serum cystatin C compared to serum creatinine in evaluating renal function, especially in the presence of early renal damage.

However, in clinical practice in Vietnam, there is some debate about which markers should be used for the diagnosis of kidney damage in patients with diabetes. As a result, we conducted this study to provide additional evidence on the validity of using cystatin C makers. In detail, this study investigated cystatin C concentration and explored the relationship between serum cystatin C concentration and other factors (serum creatinine, GFR estimated by MDRD, stages of chronic kidney disease) in DM type 2 patients with kidney damage.

2. Materials and Method

2.1. Subjects of Study

79 patients diagnosed with DM type 2 were divided into two groups (with or without kidney damage). Group 1: Patients were diagnosed with kidney damage (KD group) when they had at least one of three symptoms: GFR estimated <60 mL/minute/1.73 m^2 (GFR formula); Abnormal urine ACR index >3 mg/mmol; Abnormal proteinuria index >30 mg/day. Group 2: Patients were determined not to have kidney damage (NKD group) when they met the criteria: glomerular filtration rate ≥ 60 mL/minute (estimated based on creatinine), the ACR <3 mg/mmol and Proteinuria index of <30 mg/day, all within reference range [7].

2.2. Selection Criteria

The patients have been diagnosed with DM type 2 with clinical information (age, gender, history, medical examination) and subclinical test results (serum creatinine concentration, serum cystatin C concentration, serum glucose concentration, serum HbA1C, total analysis of urine parameters (11 parameters) and voluntarily participated in the study.

2.3. Exclusion Criteria

Patients with DM type 1, gestational diabetes, urolithiasis, or chronic pyelonephritis, treated with corticosteroids within one month before the time of study or with associated endocrine conditions (Thyroid disease, adrenal medullary tumor, pituitary gland disease).

2.4. Time and Place of Study

The study was conducted from June 2018 to January 2019 at Bach Mai Hospital, Hanoi, Vietnam.

2.5. Study Method

Prospective, descriptive, cross-sectional study.

Diagnosing diabetes according to the guidelines of the American Diabetes Association

2018 [12]. Diagnostic criteria for renal complications follow the guidelines of the 2013 National Nephrology Council [13].

Equation to estimate glomerular filtration rate: Based on creatinine: MDRD (Modification of Diet in Renal Disease) equation [7]:

MLCTcre = 186,3 × creatinine$^{-1,154}$ (mg/dL) × age$^{-0,203}$ × 0.742 (female)

Patients were examined and blood samples were taken for biochemical tests.

Testing techniques: Glucose Quantitation: Hexokinase method. Creatinine quantitation: Two-point kinetic method (AU 5800 and ARCHITECT i2000SR). Triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol quantitation: Enzyme colorimetric method. HbA1c quantitation: immunity measuring turbidity. Cystatin C quantitation: immunity to turbidity measurement (PETIA) using Architect C8000. Urinalysis was performed on a Clinitack Novus analyzer to give semi-quantitative results.

2.6. Statistical Analysis

Data were analyzed using SPSS 22.0 software (IBM, American). Analysis of variance (ANOVA), Chi-square tests ($\chi 2$) and Pearson correlation were applied to the corresponding cases, and Student's t-test was used to compare the means between the two groups. The p-value of less than 0.05 was considered a statistically significant difference.

3. Results

3.1. Characteristics of the Subjects

Our study was conducted on 79 patients, including 45 males and 34 females. The ratio of males to females was 1.3:1.

The average age and serum creatinine levels of the KD group (64.480 ± 14.114 years; 119.460 ± 101.193 µmol/L) were significantly higher than the NKD group (55.552 ± 14.017 years; 72.241 ± 14.766 µmol/L) with p = 0.009 and 0.002. There was no significant difference in serum glucose levels and HbA1c between these two groups (Table 1).

3.2. Serum Cystatin C Levels

In our study, serum cystatin C levels in the KD group increased significantly by age group (p=0.030), and there was no difference in the NKD group (p=0.094). In the KD group, serum cystatin C levels increased more than in the NKD group (2.24 times from 0.805 to 1.81 mg/L and 1.3 times from 0.805 to 1.049 mg/L) (Table 2).

Table 1. Characteristics of the subjects

Characteristics ($\bar{x}$ ± SD)	NKD group	KD group	p
Age (year)	55.552 ± 14.017	64.480 ± 14.114	0.009
Glucose (mmol/L)	12.352 ± 6.666	14.096 ± 7.691	0.311
Creatinine (µmol/L)	72.241 ± 14.766	119.460 ± 101.193	0.002
HbA1c (%)	11.203 ± 11.564	10.188 ± 3.376	0.562

Table 2. Serum cystatin C levels (mg/L) in age groups

Age (year)	<49	50-59	60-69	>70	p
KD group ($\bar{x}$± SD)	0.805 ± 0.183	1.166 ± 0.474	1.293 ± 0.725	1.810 ± 1.109	0.030
NKD group ($\bar{x}$± SD)	0.805 ± 0.157	0.898 ± 0.137	0.995 ± 0.197	1.049 ± 0.264	0.094

Table 3. Serum cystatin C levels (mg/L) in two genders

Serum cystatin C levels (mg/L)	All patients	Male	Female	p
KD group ($\bar{\boldsymbol{x}}$ ± SD)	1.40 ± 0.89	1.285 ± 0.607	1.509 ± 1.090	0.381
NKD group ($\bar{\boldsymbol{x}}$ ± SD)	0.93 ± 0.21	0.929 ± 0.224	0.925 ± 0.163	0.941

The results showed that there was no significant difference in cystatin C levels between males and females, in both the KD group and NKD group (p=0.381 and 0.941, respectively) (Table 3).

3.2. Relationship Between Serum Cystatin C Levels and Some Factors Related to Kidney Diseases

Serum creatinine and cystatin C levels have a positive correlation. The KD group was more closely correlated than the NKD group with r=0.944 and 0.462, respectively. In our study, serum cystatin C levels and $eGFR_{Cre}$ have an inverse correlation (Figure 1).

In our study, the NKD group has only patients in stages 1 and 2, with slight changes in renal function. Serum creatinine and cystatin C levels were still in the reference range. In the KD group in stages 3, 4, and 5, the creatinine and cystatin C levels increased compared to the upper limit of the reference range. In order to have appropriate treatment, it is necessary to detect kidney damage at an early stage. We, therefore, compared the increase in serum creatinine and cystatin C levels in phase 3 to the upper limit of the reference range [15, 16] (Table 4).

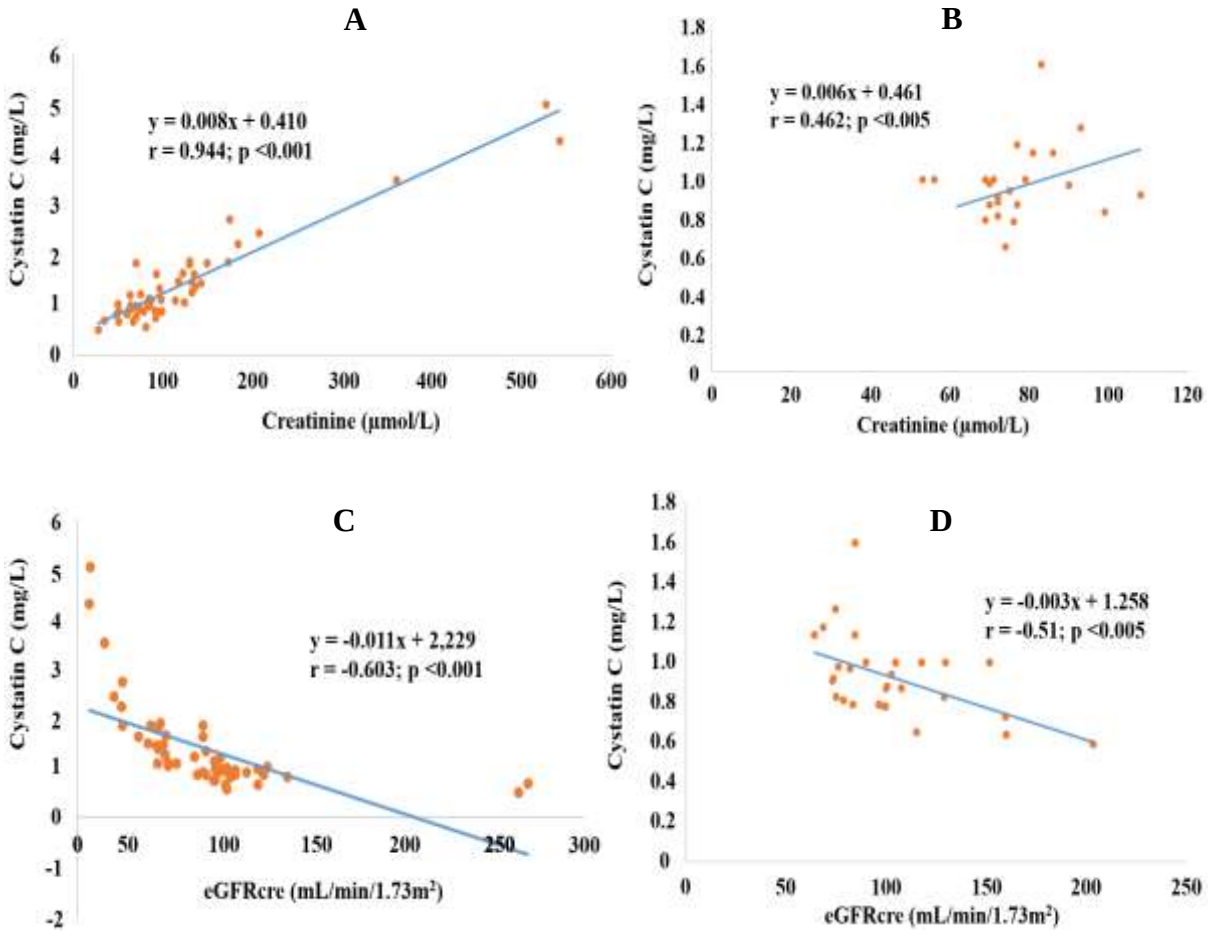

Figure 1. Correlation between serum cystatin C and creatinine levels in KD group (A) and NKD group (B), Correlation between serum Cystatin C levels and glomerular filtration rate estimated by serum creatinine levels in KD group (C) and NKD group (D).

Table 4. Serum cystatin C levels according to stages of chronic kidney disease

Stages of chronic kidney disease	1	2	3	4	5	p
NKD group	n = 16	n = 13				
Creatinine (µmol/L)	63.500 ± 10.570	83.000 ± 11.909				0.005
Cystatin C (mg/L)	0.834 ± 0.136	1.042 ± 0.225				<0.001
KD group	n = 11	n = 18	n = 14	n = 5	n = 2	
Creatinine (µmol/L)	55.455 ± 15.833	80.444 ± 12.430	124.857 ± 16.645	219.600 ± 79.670	534.500 ± 10.607	<0.001
Cystatin C (mg/L)	0.832 ± 0.152	1.034 ± 0.322	1.446 ± 0.294	2.552 ± 0.618	4.660 ± 0.890	<0.001

Table 5. Comparison of serum cystatin C and serum creatinine levels with upper range in patients in stage 3 of chronic kidney disease

Index		Patients in stage 3 of chronic kidney disease	Upper limit of normal	p
Creatinine (µmol/L)	Male	133.000 ± 8.426	120	0.002
	Female	110.200 ± 18.458	97	0.185
Cystatin C (mg/L)		1.446 ± 0.294	1.2	0.008

We found a significant difference in the increase of serum cystatin C level compared to the upper limit of the reference range (p=0.008) and creatinine levels of males (p=0.002), while there was no significant difference in creatinine levels of females (p=0.120) (Table 5).

4. Discussion

4.1. Characteristics of the Subjects

HbA1C is an indicator that evaluates the condition of chronic hyperglycemia in diabetic patients for both diagnosis and monitoring treatment. Studies have not shown the effect of daily serum glucose fluctuations on the progression of diabetic nephropathy, while HbA1C levels are associated with the risk of kidney disease [16]. NKF KDOQI recommends that HbA1C levels >7.0% are targeted for glycemic control in type 2 diabetic patients who have not progressed to end-stage chronic kidney disease [17]. It can be seen in Table 1 that the glucose and HbA1c levels between the two groups had no significant difference, demonstrating stable glycemic control in both groups.

Serum creatinine levels increased significantly in the KD group compared to the NKD group (p=0.02). The increase in creatinine levels in the KD group was an inevitable consequence of impaired renal function. In addition to the average value of creatinine levels that may reflect the degree of chronic kidney disease, the variability of creatinine levels has also been studied as a predictor of the occurrence of albuminuria [18].

4.2. Serum Cystatin C Levels

The results of our study showed that in patients without kidney damage, there was no significant difference by age group of cystatin C concentration. This concurred with Knight (2004), who found similar results regarding

cystatin C in relation to age, gender, and weight factors [19]. In the KD group, a significant increase in serum cystatin C levels by age group may be a result of a rapid decline in glomerular filtration rate in patients who already had kidney damage, compared with a gradual decrease in the NKD group.

Serum cystatin C levels between the two genders in our research showed no significant difference. Similar to other previous studies, cystatin C is independent of gender. Our results supported the advantages of cystatin C over creatinine [20].

4.3. Relationship between Serum Cystatin C Levels and Some Factors

Buysschaert et al., (2003) found that the correlation between creatinine and cystatin C plasma concentrations was 0.92 [21]. The study of Dsa et al., (2017) on 120 patients with chronic kidney disease showed this correlation with r = 0.875, p <0.001 [22]. When studied in patients in the intensive care unit, Lipcsey et al., (2011) showed similar results [23]. Although there were differences in subjects and research methods, the results showed a strong correlation between cystatin C and creatinine. This result can be explained because both substances have the same mechanism of concentration increase, the difference made by the influencing factors of each substance.

Regarding the relationship between serum cystatin C levels and $eGFR_{Cre}$, our results were similar to that of Lee (2005) in 73 diabetic patients with r=0.637 (p<0.01) (1/cystatin C and $eGFR_{Cre}$ [24]. When compared with the glomerular filtration rate accurately measured by 51Cr-EDTA, cystatin C still showed a closed correlation, and it was a better indicator than creatinine and eGFR by Cockcroft-Gault equation (0.84 compared to 0.65 and 0.7) [25].

Thus, many studies showed that cystatin C was strongly correlated with creatinine and GFR_{Cre} estimated by many equations [26]. However, for clinical practice, further research is needed to be done to apply this indicator in each hospital. Our research has contributed to the use of cystatin C instead of creatinine in cases where creatinine proved less accurate when estimating the glomerular filtration rate.

In the study of Dsa et al., (2017), serum cystatin C levels were assessed in 4 groups, one group consisted of patients with stages 1 and 2. The results showed a significant increase by stage (p<0.001). The average cystatin C concentration measured was 2.44 mg/L, higher than our study, probably due to the difference in the number of patients in each group. The study also observed that creatinine in patients with GFR of 45-60 mL/minute was still within the reference range (0.7-1.4 mg/dL), while cystatin C began increasing [22]. The result of our study in Table 5 also showed that cystatin C levels increased earlier than creatinine levels in stage 3 of chronic kidney disease of the KD group in females. Thus, our research supports serum cystatin C as an early marker of reduced glomerular filtration rate.

4. Conclusion

Serum cystatin C levels increased gradually with the stage of chronic kidney disease in Vietnamese patients with type 2 diabetic nephropathy (p < 0.001). Monitoring cystatin C levels could be a marker to predict renal function in patients.

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Conflicts of interest

None declared.

Reference

[1] S. Pouya, P. Inga, S. Paraskevi, M. Belma, K. Suvi, U. Nigel, C. Stephen, G. Leonor, M. A. Ayesha, O. Katherine, S. E. Jonathan, B. Dominic, W. Rhys, Global and Regional Diabetes Prevalence Estimates for 2019 and Projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th Ed, Diabetes Research and Clinical Practice, Vol. 157, Article 107843, 2019, pp. 0168-1277, https://doi.org/10.1016/j.diabres.2019.107843.

[2] B. Christian, S. Vera, H. Esther, M. G. Jennifer, A. Rifat, B. Till, D. Justine, V. Sebastian, Global Economic Burden of Diabetes in Adults: Projections from 2015 to 2030, Diabetes Care, Vol. 41, No. 5, 2018, pp. 963-970, https://doi.org/10.2337/dc17-1962.

[3] N. Holman, B. Young, R. Gadsby, Current Prevalence of Type 1 and Type 2 Diabetes in Adults and Children in the UK, Diabetic Medicine, Vol. 32, No. 9, 2015, pp. 1119-1120, https://doi.org/10.1111/dme.12791.

[4] Centers for Disease Control and Prevention, US Department of Health and Human Services 2017, National Center for Chronic Disease Prevention and Health Promotion, National Diabetes Statistics Report, Atlanta, 2017.

[5] D. D. Anjali, H. Marcie, S. Mario, Epidemiology of Diabetes and Diabetes-Related Complications, Physical Therapy, Vol. 88, No. 11, 2008, pp. 1254-1264, https://doi.org/10.2522/ptj.20080020.

[6] A. S. Lesley, C. Josef, G. Tom, S. L. Andrew, Assessing Kidney Function - Measured and Estimated Glomerular Filtration Rate, New England Journal of Medicine, Vol. 354, No. 23 2006, pp. 2473-2483, https://doi.org/10.1056/nejmra054415.

[7] International Society of Nephrology, Chapter 1: Definition and Classification of CKD, Kidney International Supplements 2013, KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, Vol. 3, No. 1, 2013, pp. 19-62, https://doi.org/10.1038/kisup.2012.64.

[8] O. Tenstad, A. B. Roald, A. Grubb, K. Aukland, Renal Handling of Radiolabelled Human Cystatin C in the Rat, Scandinavian Journal of Clinical and Laboratory Investigation, Vol. 56, No. 5, 1996, pp. 409-414, https://doi.org/10.3109/00365519609088795.

[9] Y. K. Jeon, M. R. Kim, J. E. Huh, J. Y. Mok, S. H. Song, S. S. Kim, B. H. Kim, S. H. Lee, Y. K. Kim, I. J. Kim, Cystatin C as An Early Biomarker of Nephropathy in Patients with Type 2 Diabetes, Journal of Korean Medical Science, Vol. 26, No. 2, 2011, pp. 258-263, https://doi.org/10.3346/jkms.2011.26.2.258.

[10] A. G. Christensson, A. O. Grubb, J. A. Nilsson, K. Norrgren, G. Sterner, G. Sundkvist, Serum Cystatin C Advantageous Compared with Serum Creatinine in The Detection of Mild but Not Severe Diabetic Nephropathy, Journal of Internal Medicine, Vol. 256, No. 6, 2004, pp. 510-518, https://doi.org/10.1111/j.1365-2796.2004.01414.x.

[11] V. C. Amanda, Q. Marian, L. F. A. Andre, S. P. Sandra, Performance of Cystatin C-Based Equations for Estimation of Glomerular Filtration Rate in Diabetes Patients: A Prisma-Compliant Systematic Review and Meta-Analysis, Scientific Reports, Vol. 9, No. 1418, 2019, https://doi.org/10.1038/s41598-018-38286-9.

[12] American Diabetes Association, Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes, Diabetes Care, Vol. 42, Suppl. 1, 2019, pp. S13-S28, https://doi.org/10.2337/dc19-s002.

[13] S. Chen, C. Tseng, Dyslipidemia, Kidney Disease, and Cardiovascular Disease in Diabetic Patients, The Review of Diabetic Studies, Vol. 10, No. 2-3, 2013, pp. 88-100, https://doi.org/10.1900/rds.2013.10.88.

[14] E. Lim, J. Miyamura, J. J. Chen, Racial/Ethnic-Specific Reference Intervals for Common Laboratory Tests: A Comparison Among Asians, Blacks, Hispanics, and White, Hawaii J Med Public Health, PMID: 26468426; PMCID: PMC4578165, Vol. 74, No. 9, 2015, pp. 302-310.

[15] A. Ognibene, E. Mannucci, A. Caldini, A. Terreni, M. Brogi, G. Bardini, I. Sposato, V. Mosconi, B. Salvadori, C. M. Rotella, G. Messeri, Cystatin C Reference Values and Aging, Clinical Biochemistry, Vol. 39, No. 6, 2006, pp. 658-661, https://doi.org/10.1016/j.clinbiochem.2006.03.017.

[16] J. M. Richard, J. George, I. E. Elif, Effects of Glycaemic Management on Diabetic Kidney Disease, World Journal of Diabetes, Vol. 8, No. 5, 2017, pp. 172-186, https://doi.org/10.4239/wjd.v8.i5.172.

[17] T. S. Park, How Much Glycemic Control Is Needed to Prevent Progression of Diabetic Nephropathy?, Journal of Diabetes Investigation, Vol. 3, No. 5, 2012, pp. 411-412, https://doi.org/10.1111/j.2040-1124.2012.00225.x.

[18] L. Serena, X. Zhang, K. Ang, J. D. Y. Su, J. L. Guanyi, L. Y. Yeoh, Y. L. Lin, S. Tavintharan, C.F. Sum, S.C. Lim, Discovery and Validation of Serum Creatinine Variability as Novel Biomarker for Predicting Onset of Albuminuria in Type 2 Diabetes Mellitus, Diabetes Research and Clinical Practice, Vol. 138, 2018, pp. 8-15, https://doi.org/10.1016/j.diabres.2017.11.003.

[19] L. K. Eric, C. V. Jacobien, S. Donna, L. H. Hans, Z. Dick, C. C. Gary, E. J. Paul, Factors Influencing Serum Cystatin C Levels Other than Renal Function and the Impact on Renal Function Measurement, Kidney International, Vol. 65, No. 4, 2004, pp. 1416-1421. https://doi.org/10.1111/j.15231755.2004.00517.x.

[20] O. A. Musaimi, A. H. A. Nawwas, D. A. Shaer, N. Y. Khaleel, M. Fawzi, Influence of Age, Gender, Smoking, Diabetes, Thyroid and Cardiac Dysfunctions on Cystatin C Biomarker, Medicina de Familia. SEMERGEN, Vol. 45, No. 1, 2019, pp. 44-51, https://doi.org/10.1016/j.semerg.2018.07.005.

[21] M. Buysschaert, I. Joudi, P. Wallemacq, M. P. Hermans, Comparative Performance of Serum Cystatin – C Versus Serum Creatinine in Diabetic Subjects, Diabetes & Metabolism, Vol. 29, No. 4, 2003, pp. 377-383, https://doi.org/10.1016/s1262-3636(07)70048-4.

[22] D. Janice, S. Sukanya, R. B. Roopa, V. R. Ashalatha, Association Between Serum Cystatin C and Creatinine in Chronic Kidney Disease Subjects Attending a Tertiary Health Care Centre, Journal of Clinical and Diagnostic Research, Vol. 11, No. 4, 2017, pp. BC09-BC12, https://doi.org/10.7860/JCDR/2017/26655.9655.

[23] L. Miklos, F. Mia, R. Sten, L. Anders, Significant Differences When Using Creatinine, Modification of Diet in Renal Disease, or Cystatin C for Estimating Glomerular Filtration Rate in ICU Patients, Ups J Med Sci, Vol. 116, No. 1, 2011, pp. 39-46, https://doi.org/10.3109/03009734.2010.526724.

[24] S. H. Lee, G. Y. Ahn, O. Y. Jeong, Y. J. Park, S. J. Jang, D. S. Moon, Usefulness of Serum Cystatin C for the Evaluation of Renal Function in Diabetic Patient, Korean J Lab Med, Vol. 25, No. 3, 2005, pp. 155-161.

[25] M. Michele, D. V. Michele, F. Paola, S. Alois, V. Mariacristina, N. Romano, P. Mario, Cystatin C is a More Sensitive Marker than Creatinine for the Estimation of GFR in Type 2 Diabetic Patients, Kidney International, Vol. 61, No. 4, 2002, pp. 1453-1461, https://doi.org/10.1046/j.15231755.2002.00253.x.

[26] R. D. Vikas, K. Charles, S. Gary, Serum Cystatin C is Superior to Serum Creatinine As a Marker of Kidney Function: A Meta-Analysis, American Journal of Kidney Diseases, Vol. 40, No. 2, 2002, pp. 221-226, https://doi.org/10.1053/ajkd.2002.34487.