Received: 04 December 2021 | Revised: 21 January 2022 | Accepted: 28 January 2022
DOI: 10.22034/ecc.2022.322991.1288
Eurasian Chemical Communications
SAMI

FULL PAPER

Crucial biomarkers for early detection of chronic kidney disease; Neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-6 (IL)-6

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Chronic kidney disease (CKD) is a gradually progressive disease that recently assumed epidemic proportion. Recent studies suggested that both Neutrophil gelatinase-associated lipocalin (NGAL) produced by tubular cells of the kidney and interleukin 6 (IL-6) cytokine are involved in the pathogenesis of CKD. However, for this reason, the research aimed to assess whether (NGAL) and IL-6 could predict the early detection of CKD. Serum and urinary NGAL and IL-6 levels for all healthy and CKD patients were tested via enzyme-linked immunosorbent assay (ELISA). The result notably showed that both serum NGAL and urinary NGAL levels were significantly increased with disease severity. In the other hand, this study reported that serum IL-6 and urine IL-6 in patients with CKD were significantly increased compared with the control group. This study suggests that serum and urine of both NGAL and IL-6 might be a potentially beneficial biomarker for the early diagnosis of CKD. In conclusion, the level of NGAL and IL-6 is significantly increased in CKD, indicating that NGAL and IL-6 are good indicators for early detection of CKD.

KEYWORDS

Chronic kidney disease; interleukin-6; neutrophil gelatinase-associated lipocalin.

Introduction

Chronic kidney disease (CKD), also called chronic kidney failure, is a general term for a disorder altering both structure and function of the kidney that often progresses to end-stage kidney failure. This disease is diagnosed by measuring the level of creatinine in the blood and urine. There are few methods to diagnose CKD, such as urine dipstick, which is a rapid point of care and inexpensive. This is widely used in CKD screening. An alternative method to identifying CKD is measuring elevated albumin/creatinine ratio. These methods are susceptible in the early stages of protein-uric CKD; However, the applicability has limitations in diagnosing early CKD, owing to the minimally protein-uric nature of this disease [1]. Of note, a previous study has revealed that levels of serum creatinine increase only when renal parenchymal damage reaches 40% to 50% and above, therefore, rendering both serum creatinine and the estimating equations (estimated glomerular filtration rate) on which it is based a late marker of renal damage. This suggests that the serum creatinine method may not be ideal for identifying early kidney damage [1]. Human Neutrophil gelatinase-associated lipoxin (NGAL) identified initially as 25 kDa protein belonging to the lipocalin family bound to gelatinase from neutrophils, has got attention in the past few years as an early marker of kidney problems, with its levels in the blood and urine rising much earlier than serum creatinine levels [2]. NGAL is better at

diagnosing kidney problems than creatinine [3, 4]. As its level rises early before creatinine rises, this contributes to early detection [5]. Interleukin-6 (IL-6) is a pleiotropic cytokine, a 26 kDa protein released from the liver. It plays a crucial role in the acute-phase of the inflammatory response, B-cell proliferation, and differentiation, promoting lymphocyte activation, recruitment of leukocyte, and regulating the synthesis of acute-phase proteins fibrinogen and albumin [4,6]. In this research, we assessed the diagnostic value of urine and serum of both IL-6 and NGAL for CKD patients to identify sensitive and specific biomarkers for the early diagnosis of CKD.

Materials and methods

Subjects

This study was carried out on 45 patients with CKD and 45 healthy subjects with age range (35 to 67) years. Controls were selected as non-diabetic, non-hypertensive, clear from acute diseases

Also, there was no record of drinking or consuming alcohol. Patients were selected randomLy from Baghdad Teaching Hospital/Medical City. Blood and urine samples were collected, and a well-structured questionnaire (age, gender, smoking, and BMI) was filled in after a complete clinical examination via certified consultants. Chronic kidney patients were diagnosed by the amount of glomerular filtration rate (glomerular filtration rate) and albumin/creatinine ratio (ACR) (stage I, stage II, stage IIIA, stage IIIB)

NGAL and IL-6 Detection

For measure NGAL and IL-6, blood samples were collected from all subjects. Samples were centrifuged at 3500 r/min for 10 minutes, then frozen at -80 °C for later detection. Urine samples were collected from all subjects 24 hours after the URL.

NGAL and IL-6 levels were tested in both serum and urine samples by enzyme-linked immunosorbent assay (ELISA), kits were provided by Melsin (China). Urea, uric acid, albumin, and serum creatinine were measured through a spectrophotometer.

Statistical analysis

Statistical analysis was accomplished using SPSS version 22.0. Data expressed as means ±SD. The difference in the mean of a normal distribution quantitative variable was evaluated through an analysis variance T-Test. *P*-values less than 0.05 were considered significant for IL-6 and NGAL levels.

Result and discussion

Table 1 shows the mean values (± SD), the extent and distribution of the studied groups (number and percentage), age (in years), gender, and smoking and disease stages.

The mean values of ± SD for the age of the healthy group and the chronic kidney disease include (50.689±7.954), (53.311±9.798), respectively, the results were not significant between the groups (P > 0.05) between the mean values of (± SD) for age in the studied groups. However, we note that patients with chronic kidney disease were the most common among the elderly, as the older a person is, the more likely he is to develop diseases [7].

Gender ratio of the two groups was as follows, (48.2758% for male and 50.819% for female) and (51.724% for male and 49.180% for female) for the healthy group and the chronic kidney disease, respectively, the result, no significant (P >0.05) difference between the sex ratio in the studiedgroups, men were higher than women. Men are more likely to have acute renal insufficiency than women [8]. Smoking outcomes were not significant (P >0.05). Smoking is the leading cause of many diseases. This study noted that patients' groups' percentages were higher than healthy people [9]. While the stage ratio

in the studied groups, the result were high significant ($p < 0.05$) difference between the stage ratio in the studied groups. The stages are based on the eGFR test result and how well your kidneys filter waste and extra fluid out of your blood. As the stages go up, kidney disease worsens, and your kidneys do not work. According to risk factors and stage of disease, there are marked variations in the time spent in the different stages of CKD [10].

TABLE 1 The mean (± SD) values and Distribution (number and %) for some epidemiological variables and healthy groups with chronic kidney disease

variables	Cats.	G1		G2		Total		P value
		N	%	N	%	N	%	
Age (years) [b]		50.689± 7.954		53.311±9.798		52± 8.97		0.051 NS
Gender [a]	M	14	48.275	0.822 NS	51.724	29	32.22	0.822
	F	31	50.8196		49.180	61	67.78	NS
Smoking [b]	0	35	51.4705	0.624 NS	48.53	68	75.56	0.624
	1	10	45.454		54.545	22	24.44	NS
Stage [c]	I	45	100.00	0.000 *	0.00	45	50.00	0.000 *
	II	0	.00	9	100.00	9	10.00	
	IIIa	0	.00	18	100.0	18	20.00	
	IIIb	0	.00	18	100.0	18	20.00	

[a]=chi square [b] =Tow sample T test, [c] = Fisher exact *=significant at p<0.05

In Table 2, the results are significant ($p < 0.05$), as the Body Mass Index (BMI) for CKD patients, was higher than healthy people, and this confirms that the higher the BMI, the more susceptible the person to diseases, and we also noted that the Waist-to-Hip Ratio (WHR) was not significant($p > 0.05$), it is higher in the CKD patients than in healthy people, where the higher the WHR was The susceptible to disease [11].

TABLE 2 Mean values (±SD) and distribution (number and percentage) of BMI and WHR variables for patient groups with healthy groups and their significance

	Groups	Min	Max.	Mean	±SD	P value
BMI	Healthy	17.306	39.136	27.524	6.160	0.032 *
	CKD	19.928	73.217	31.542	10.744	
WHR	Healthy	0.700	1.010	0.832	0.118	0.082 ^
	CKD	0.735	0.990	0.873	0.104	

^=not significant, *=significant at p<0.05.

In Table 3, the results are significant ($p < 0.05$) for the parameters (Uric Acid, Urea, and s.cr) that all of these parameters increased in CKD patients. While albumin-to-creatinine ratio ACR and eGFR were significant ($p < 0.05$) results as ACR increased in patients CKD, while eGFR decreased in patients because the filtration process decreases when the kidneys are damaged. In CKD patients, it has been observed that renal excretion of uric acid is minimized, resulting in hyperuricemia [12]. Studies showed that increased creatinine levels and renal impairment based on eGFR levels has been associated with an increased risk of renal failure. Creatinine is freely filtered by the glomerulus at a stable rate but released by the tubules (10% to 40%). The tubular secretion of creatinine increases with CKD leading to an unpredictable overestimation of GFR [13]. It has been suggested that absolute blood urea can be predictive for developing kidney disease. These results may indicate that the effect of blood urea level on the progression of kidney disease is more substantial in the early stages of CKD [14]. It was found that a decrease in eGFR in CKD and

non-CKD patients should be evaluated in terms of accident risk and all-cause mortality because the eGFR slope in CKD with eGFR <60 mL/min/1.73 m^2 is further significant and sensitive than eGFR >60 mL/min/1.73 m^2 [14].

TABLE 3 Mean ± SD and range of renal function parameter for the Healthy and chronic kidney disease groups

	Groups	Minimum	Maximum	Mean	±SD	P value
Uric acid	Healthy	3.100	6.120	4.377	1.186	0.000*
	CKD	3.000	9.520	6.702	1.536	
Urea	Healthy	17.000	32.000	22.867	4.855	0.000*
	CKD	11.000	216.000	76.800	46.237	
s.cr	Healthy	0.510	0.840	0.661	0.114	0.000*
	CKD	0.620	2.850	1.580	0.564	
Albumin-Creatinine ratio (ACR)	Healthy	4.545	27.397	14.143	8.926	0.000*
	CKD	10.101	612.245	132.381	152.981	
e GFR	Healthy	90.749	145.860	113.768	19.821	0.000*
	CKD	17.661	107.436	45.323	20.829	

*=significant at p<0.05, ^=not significante significance level was obtained at (P<0.000).

Heerspink *et al*. (2019) revealed in patients with CKD, increased proteinuria (or albuminuria) is a notable predictor of CKD progressions [15]. In Table 4, the results were significant (p<0.05) for (NGAL and IL-6) in serum and urine, as the level of (NGAL and IL-6) rises when kidney damage or inflammation occurs. With disease severity, urinary NGAL quantity was remarkably increased. This result is consistent with another study that stated that a higher serum and urinary NGAL was higher in patients with CKD than healthy subjects. Therefore, NGAL would represent The expression of how much active kidney damage lies beneath the overall condition of chronic renal impairment, rather than being a simple marker of reduced filtration such as serum creatinine, that NGAL could serve as a marker of renal function with CKD [16]. The level of IL-6 increases as CKD progresses with the rise, becoming outstanding at CKD stages 5 and 5D [6]. ROC analysis enabled the assessment of NGAL in serum usefulness as the predictor of progression in the analyzed patients CKD with the healthy group. A NGAL concentration equal to 120 ng/mL was established to area under the Curve (0.964 Area). That means the test value higher than 120 ng/mL represents the abnormal case (CKD), whereas the value that is less than 120 ng/mL considers a healthy condition, as shown in Figure 1 and Table 5.

TABLE 4 Mean ±SD value and their range of NGAL (serum and urine) and IL-6(serum and urine) for Healthy and Kidney chronic groups

	Groups	Minimum	Maximum	Mean	±SD	P value
NGAL Ser	Healthy	2.212	6.318	4.649	0.963	0.000*
	CKD	4.280	122.200	105.659	33.387	
NGAL U	Healthy	2.661	7.281	5.200	0.917	0.000*
	CKD	4.791	127.500	106.757	33.489	
IL-6 Ser	Healthy	1.223	14.010	3.814	3.741	0.000*
	CKD	9.180	48.800	44.091	9.428	
IL-6 U	Healthy	1.683	8.218	3.482	2.030	0.000*
	CKD	2.542	49.010	42.696	11.168	

*=significant at p<0.05., ^=not significant at p >0.05

ROC analysis further demonstrated that NGAL was a sensitive indicator for the stages of CKD, suggesting that serum NGAL might be a potentially useful biomarker for early diagnosis of CKD [17, 18]. In NGAL in urine, usefulness as the predictor of progression in the analyzed patients CKD with the healthy group. NGAL concentration equal to 120 ng/mL was established under the Curve (0.961 Area).

That means, the test value higher than 120 ng/mL represents the abnormal case (CKD), whereas the value that less than 120 ng/mL consider a healthy condition as shown in Figure 1 and Table 5 Patel *et al*. 2015, Yi A, *et al*. 2021 [16, 19]. The significance level was obtained at (P<0.000).

ROC analysis showed that NGAL was a sensitive indicator for the stages of CKD, suggesting that urine NGAL might be a potentially useful biomarker for the early diagnosis of CKD. IL-6 in serum analysis, an area under the ROC curve (AUC) is (0.994). Value IL-6 48 pg/mL accordingly, test value higher than 48 Pg/mL considered abnormal case whereas above this value represented healthy as shown in Figure 1 and Table 5. The significance level is obtained at (P<0.000). ROC analysis demonstrated that IL-6 was a sensitive indicator for the stages of CKD, suggesting that serum IL-6 might be a potentially useful biomarker for the early diagnosis of CKD. IL-6 in urine analysis, the area under the ROC curve (AUC) is (0.979). Value of IL-6 48 pg/mL accordingly, test value higher than 48 Pg/mL is considered abnormal case whereas above this value represented the healthy subjects as shown in Figure 1 and Table 5. The significance level is obtained at (P<0.000). ROC analysis further demonstrated that IL-6 was a sensitive indicator for the stages of CKD, suggesting that urine IL-6 might be a potentially useful biomarker for the early diagnosis of CKD.IL-6 level is elevated in CKD [6, 20]. However more future studies are needed.

TABLE 5 ROC for NGAL (serum and urine) and IL-6(serum and urine) between CKD group and healthy group

Groups	Area Under the Curve			
	Test Result Variable(s)	Area	Asymptotic Sig.	
Control from CKD	of NGAL ser (ng/mL)	0.964	0.000	Sig.
Control from CKD	of NGAL U (ng/mL)	0.961	0.000	Sig.
Control from CKD	of IL-6 ser (pg/mL)	0.994	0.000	Sig.
Control from CKD	of IL-6 U (pg/mL)	0.979	0.000	Sig.

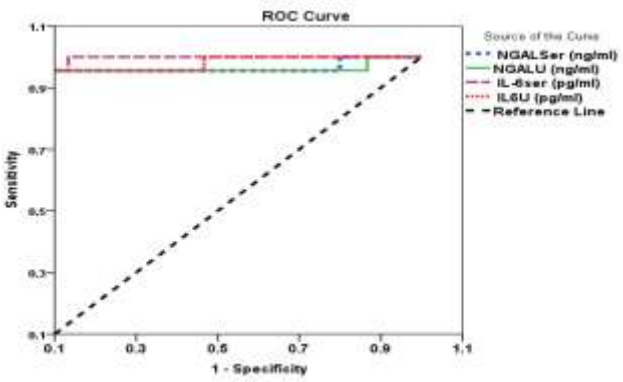

FIGURE 1 ROC curve showing the trade-off between sensitivity (rate of true positive) and rate of false positive (1-specificity) for NGAL (serum and urine) and IL-6 (serum and urine) concentration when used to predict cases with CKD differentiating them from healthy controls

Conclusion

The level of both NGAL and IL-6 is significantly increased in CKD. We note that NGAL and IL-6 are excellent indicators of early detection of CKD because they promise vital signs for kidney disease. However, further investigations with many patients enrolled for a more extended observation period are required to confirm previous findings in CKD.

Acknowledgements

The authors extend their thanks to Baghdad Teaching Hospital / Medical City and everyone who contributed to the delivery of the research.

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References

[1] B.N.T.W. Fernando, A. Alli-Shaik, R.K.D. Hemage, Z. Badurdeen, T.W. Hettiarachchi, H.T.K. Abeysundara, T.D.J. Abeysekara, A. Wazil, S. Rathnayake, J. Gunaratne, N. Nanayakkara, *Kidney Int. Rep.*, **2019**, *4*, 1401-1411. [crossref], [Google Scholar], [Publisher]

[2] D. Bolignano, V. Donato, G. Coppolino, S. Campo, A. Buemi, A. Lacquaniti, M. Buemi, *Am. J. Kidney Dis.*, **2008**, *52*, 595-605. [crossref], [Google Scholar], [Publisher]

[3] H.S Hamdy, A. El-Ray, M. Salaheldin, M. Lasheen, M. Aboul-Ezz, A.S Abdel-Moaty, A. Abdel-Rahim, *Ann. Hepatol.*, **2018**, *17*, 624-630. [crossref], [Google Scholar], [Publisher]

[4] C. Albert, M. Haase, A. Albert, M. Ernst, S. Kropf, R. Bellomo, S. Westphal, R.C. Braun-Dullaeus, A. Haase-Fielitz, S. Elitok, *Ann. Lab. Med.*, **2021**, *41*, 357-365. [crossref], [Google Scholar], [Publisher]

[5] A.M.A. Zeid, D.Y. Mohammed, A.S. AbdAlazeem, A.S. Seddeeq, A.M. Elnaany, *J. Clini. Nephrol.*, **2019**, *3*, 93-99. [crossref], [Google Scholar], [Publisher]

[6] D.V. Barreto, F.C. Barreto, S. Liabeuf, M. Temmar, H.D. Lemke, C. Tribouilloy, G. Choukroun, R. Vanholder, Z.A. Massy, *Kidney Int.*, **2010**, *77*, 550-556. [crossref], [Google Scholar], [Publisher]

[7] A. Ji, C. Pan, H. Wang, Z. Jin, J.H. Lee, Q. Wu, Q. Jiang, L. Cui, *Int. J. Environ. Res. Public Health*, **2019**, *16*, 4383. [crossref], [Google Scholar], [Publisher]

[8] G. Cobo, M. Hecking, F.K. Port, I. Exner, B. Lindholm, P. Stenvinkel, J.J. Carrero, *Clin. Sci.*, **2016**, *130*, 1147-1163. [crossref], [Google Scholar], [Publisher]

[9] F.O. Coelho, L. Andrade, *Contrib. Nephrol.*, **2021**, *199*, 179-187. [crossref], [Google Scholar], [Publisher]

[10] E. Ku, K.L. Johansen, C.E. McCulloch, *Clinical J. Am. Soc. Nephrol.*, **2018**, *13*, 693-701. [crossref], [Google Scholar], [Publisher]

[11] A. Guligowska, A. Corsonello, M. Pigłowska, R. Roller-Wirnsberger, G. Wirnsberger, J. Ärnlöv, A.C. Carlsson, L. Tap, F. Mattace-Raso, F. Formiga, R. Moreno-Gonzalez, E. Freiberger, C. Sieber, P.G. Gregorio, S.L. Martínez, R. Artzi-Medvedik, I. Yehoshua, P. Fabbietti, F. Lattanzio, T. Kostka, *BMC geriatrics*, **2020**, *20*, 1-12. [crossref], [Google Scholar], [Publisher]

[12] T.R. Oh, H.S. Choi, C.S. Kim, E.H. Bae, S.K. Ma, S.A. Sung, Y.S. Kim, K.H. Oh, C. Ahn, S.W. Kim, *Sci. Rep.*, **2019**, *9*, 1-9. [crossref], [Google Scholar], [Publisher]

[13] M. Seki, M. Nakayama, T. Sakoh, R. Yoshitomi, A. Fukui, E. Katafuchi, S. Tsuda, T. Nakano, K. Tsuruya, T. Kitazono, *BMC nephrology*, **2019**, *20*, 1-10. [crossref], [Google Scholar], [Publisher]

[14] K. Nagai, C. Iseki, K. Iseki, M. Kondo, K. Asahi, C. Saito, R. Tsunoda, R. Okubo, K. Yamagata, *PloS One*, **2019**, *14*, e0216432. [crossref], [Google Scholar], [Publisher]

[15] H.J Heerspink, T. Greene, H. Tighiouart, R.T. Gansevoort, J.Coresh, A.L. Simon, T.M. Chan, F.F. Hou, J.B. Lewis, F. Locatelli, M. Praga, F.P. Schena, A.S. Levey, L.A. Inker, *The lancet Diabetes & endocrinology*, **2019**, *7*, 128-139. [crossref], [Google Scholar], [Publisher]

[16] M.L. Patel, R. Sachan, R. Misra, R. Kamal, R. Shyam, P. Sachan, *Int. J. Nephrol. Renovasc. Dis.*,

2015, *8*, 139-144. [crossref], [Google Scholar], [Publisher]
[17] L. Lupu, H. Abukatash, A. Banai, K.L. Rozenfeld, D. Lewit, I. Merdler, I. Loewenstein, G. Bornstein, S. Banai, Y. Shacham, *J. Clin. Med.*, **2021**, *10*, 5403. [crossref], [Google Scholar], [Publisher]
[18] W.H. Lim, J.R. Lewis, G. Wong, R. Teo, E.M. Lim, E. Byrnes, R.L. Prince, *Am. J. Nephrol.*, **2015**, *41*, 156-164. [crossref], [Google Scholar], [Publisher]
[19] A. Yi, C.H. Lee, Y.M. Yun, H. Kim, H.W. Moon, M. Hur, *Ann. Lab. Med.*, **2021**, *41*, 60-67. [crossref], [Google Scholar], [Publisher]
[20] H. Su, C.T. Lei CT, C. Zhang, *Front. Immunol.*, **2017**, *8*, 405. [crossref], [Google Scholar], [Publisher]

How to cite this article: Baydaa Hamad Obid Saleh*, Fayhaa M. Khaleel. Crucial biomarkers for early detection of chronic kidney disease; Neutrophil gelatinase-associated lipocalin (NGAL) and Interleukin-6 (IL)-6. *Eurasian Chemical Communications*, 2022, 4(3), 272-278. **Link:** http://www.echemcom.com/article_144497.html