Diagnostic value of serum cystatin C and creatinine in early detection of renal failure

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Abstract

Background: Renal failure is a serious disease and pandemic health challenge, and its development to end-stage renal failure can be delayed or prevented by early diagnosis. Serum markers for detecting early renal impairment include creatinine, cystatin C, β-microglobulin and others.

Objectives: To assess the potential role of serum markers for early detection of renal failure in Hail population, Saudi Arabia.

Methods: Serum levels of cystatin C, creatinine and other factors were measured in 135 renal failure patients and 150 controls. Receiver operating characteristic (ROC) curve analysis was performed to assess the utility of biomarkers for early diagnosing renal impairment (RI).

Results: The area under the ROC curve (AUC) for serum cystatin C (Cys C) (0.946) was significantly higher than that of creatinine (0.907, $p = 0.048$), which indicates Cys C to be a better biomarker for early detection of RI compared to the commonly used serum creatinine. However, serum creatinine was found to be superior to urea (AUC = 0.727, $p < 0.01$) and uric acid (AUC = 0.619, $p < 0.01$). When serum Cys C and serum creatinine were simultaneously considered i.e. either marker was positive, the sensitivities were 98.4%, 98.6% and 98.5% for males, females and total patient group, respectively. The specificity and positive predictive value increased to 100% for the all mentioned situations.

Conclusion: Taking together, the study demonstrated that serum cystatin C is a valuable marker for early detection of renal impairment, but was found to be much more valuable when analyzed with serum creatinine.

Key words: Cystatin C, creatine, ROC, renal impairment, early detection.

Introduction

The lack of sensitive and specific markers for the detection of RI in its early stages ceases: screen patients at risk for renal injury, prognostic information on the course of RI, and monitoring the response to therapy. The use of serum creatinine suffers from delay period for its increase in early RI and is affected by age, sex, race, and muscle mass (1).

Therefore, many other biological markers have emerged with the goal of early detecting RI, including cystatin C (2), neutrophil gelatinase-associated lipocalin (3), kidney injury molecule-1 (4), and interleukin-18 (5).

Cys C is 13-kDa cysteine protease inhibitor that is produced by all nucleated cells regularly. Cys C is freely filtered by the glomeruli and catabolized by the proximal tubules without secretion; thus, it is a good marker for estimating glomerular filtration rate (GFR) (6-8). Cys C serum concentration appears to be independent of sex, age, race or muscle mass and its determination is not affected by increased bilirubin or haemolysis (9, 10). Furthermore, Cys C in healthy controls has very low variation between individuals which argues against a significant impact of diet on serum Cys C concentrations (11).

In addition, some studies comparing the area under the ROC curve for serum Cys C and serum creatinine have shown superiority for the former in predicting GFR (12-15). On the other

hand, some investigators failed to detect a significant difference between Cys C and serum creatinine in cirrhotic patients (16, 17).

A few studies have been performed in Saudi population aimed at determining the role of Cys C and creatinine in early detecting of RI including the study of Al Wakeel *et al*, (18) who showed normal range of cystatin C in a healthy subgroup of the Saudi population. Safdar *et al*, (19) however, concluded higher sensitivity of serum Cys C than creatinine, as a marker for diagnosis of acute kidney injury among children. No study yet demonstrated the simultaneous use of Cys C and creatinine for early detection of chronic renal impairment in Saudi Arabia, therefore, the aim of this study was to investigate the diagnostic value of serum Cys C simultaneously measured with creatinine in patients with renal failure and controls to early detect RI among Hail population, KSA.

Material and Methods

Study population

A total of 135 hemodialysis patients from the outpatient clinics of King Khaled hospital, Hail, KSA were included in this study in addition to 150 healthy control volunteers (sex and age-matched).

Sample collection

Serum samples were collected by medical care professionals at the King Khaled Hospital at Hail, KSA following a standard protocol.

Sample pre-treatment blood

This work was conducted on the outcome of dialysis study, which is a retrospective study as 135 blood samples were obtained from chronic kidney failure patients and 150 healthy adult

volunteers. Informed consent was obtained from all patients and controls prior to inclusion in this study. The protocol was approved by the ethics committee of the University of Hail.

Data collection

Demographic and clinical data including age, gender and clinicopathological features of the investigated patients and controls were collected

Estimation of renal function tests

Creatinine was analysed by a rate blanked modified Jaffé method (20). Urea was determined using a kinetic urease method followed by a GLDH-UV test where the decrease in NADH absorbance was determined photometrically (21). Both assays were implemented on a Hitachi multianalyser system. Serum cystatin C was measured by latex based turbidimetric immunoassay. This test uses latex particles coated with anti-human cystatin C, which form a complex with cystatin C present in a blood sample. This complex formation results in a change in turbidity, which was measured in automated clinical chemistry analyzer (22, 23).

Statistical Analysis

Data were analyzed using SPSS version 23. Data were expressed as mean ± SD. The student t-test was used to compare the mean of two groups. Bivariate correlations were tested using Pearson's (r) correlation coefficient. The ability of the studied tests and formulae to discriminate early renal impairment was evaluated using the area under the ROC curve (AUC). Two-tailed p values were considered statistically significant if they were less than 0.05. AUCs were compared using Hanly method (24). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were calculated according to the following formulae:

Sensitivity = a/(a+c)

Specificity = d/(b+d)

Positive predictive value = a/(a+b)

Negative predictive value = c/(c+d)

Positive likelihood ratio = sensitivity /(1-specificity)

Negative likelihood ratio = (1- sensitivity) /specificity

Where: a = true positive cases, c = false negative cases,

d = true negative cases, b = false positive cases.

Results

Demographic characteristics of patients and controls

The present study was performed on a total of 135 hemodialysis patients from the outpatient's clinics at the King Khaled hospital, Hail, KSA including 62 males (46%) and 73 females (54%) with a mean age of 51.3 ± 19.4 yrs. The control group consisted of 150 healthy adults including 99 males (66%) and 51 females (34%) with a mean age of 37.4 ± 14.9 yrs.

Kidney function parameters of patients and controls

Cystatin C mean level in the patient group was 2.43 ± 1.24 mg/l while in the control group it was 0.75 ± 0.14 mg/l ($p < 0.01$). All kidney function parameters including creatinine, urea and uric acid showed significant increase in patients than controls ($p < 0.05$ in all cases, Table 1).

ROC analysis of the studied parameters

The discriminating ability of the studied markers in detecting early renal impairment was assessed by plotting the ROC curves (Fig.1a-c). Table 2 presents the AUC for each of the studied parameters. Cystatin C had the largest AUC (0.946, 95% CI 0.918 - 0.975) for the patient group as a whole. Regarding the gender, the AUC was 0.963 (95% CI 0.932 - 0.995) and 0.920 (95%

CI 0.686 - 0.972) for males and females, respectively. For creatinine, it was more sensitive in detection of renal impairment in females (AUC of 0.954, CI 0.916 - 0.992) than in males (AUC of 0.863, 95% CI 0.797 - 0.930, $p < 0.001$) whereas in the patient group as a whole, the AUC was 0.907 (95% CI of 0.869 - 0.944). For urea, a lower AUC was calculated (0.727, 95% CI 0.666 - 0.787) and for uric acid the lowest AUC in all parameters was calculated (0.619, 95% CI 0.553 - 0.684).

Diagnostic criteria of the studied markers

Cystatin C

After ROC curve analysis, we revealed the optimal cut off value which is the nearest point to the upper left corner that satisfies maximum sensitivity and maximum specificity. For cystatin C (Fig. 2), an optimum cut off level of 1.11 mg/l for males and females separately and in combination as a whole patient group, at which the sensitivity was 88.71%, 82.20% and 85% for males, females and for total patients, respectively with a specificity of 100% in all. The positive predictive value (PPV) was 100% in all whereas the negative predictive values (NPV) were 93.40%, 79.69% and 88.24%, respectively. As for the likelihood ratios (LR) positive and negative, the PLR was indefinite due to division by zero and the NLR was 0.11, 0.18 and 0.15 for males, females and total patients, respectively (Table 3).

Creatinine

Due to the difference in males and females with respect to creatinine level, different optimal cut off values were determined through ROC analysis. For males, 2.20 mg/ml was calculated as the nearest point to the upper left corner of a ROC plot at which maximum sensitivity (69.35%) and maximum specificity (100%) were obtained. The PPV and NPV were 100% and 83.90%, respectively whereas the PLR was infinite and NLR was 0.31. For females,

the optimum cut off was 1.25 mg/ml at which the sensitivity, specificity, PPV and NPV were 91.8%, 96%, 97.1% and 89.09, respectively. The PLR and NLR were 22.9 and 0.09, respectively. For the patient group as a whole, a cut off of 1.98 mg/ml was determined at which the sensitivity and specificity were 77% and 99.4%, respectively. The PPV, NPV, PLR, and NLR were 99.05%, 81.21%, 128.33 and 0.23, respectively (Table 3 & Fig. 3).

Simultaneous determination of cystatin C and creatinine

When the sensitivity of cystatin C and creatinine were simultaneously considered i.e. either marker is positive, the sensitivity of both markers increased to 98.4%, 98.6% and 98.5% for males, females and total patients, respectively. The specificity and PPV increased to 100% for all situations. The NPV increased also to 99%, 98.6% and 98.5%, respectively. The PLR was not infinite (division by zero) and the NLR was 0.016, 0.014 and 0.015, respectively (Table 3 & Figs. 4, 5).

Urea and uric acid

As described for cystatin C and creatinine, the diagnostic criteria for urea and uric acid are summarized in table 3 where low sensitivity for both urea (57.55%) at cut off of 17.55 mmol/l and uric acid (48%) at a cut off of 6.31mg/dl with a good to moderate specificities of 85.7% and 71.4% were obtained respectively. The predictive values and likelihood ratios are summarized in table 3.

Correlation between the studied parameters

Table 4 summarizes the correlation between cystatin C, creatinine, urea and uric acid. Cystatin C was not correlated to any of the other parameters ($p > 0.05$) in all. Significant correlations were detected between creatinine and urea ($r = 0.680$, $p < 0.01$) creatinine and uric

acid ($r = 0.264$, $p < 0.01$) as well as between urea and uric acid ($r = 0.358$, $p < 0.01$) in the patient group as a whole. The same correlations were summarized in table 4 for males and females separately (Fig. 6).

Table 1. Demographic characteristics and kidney function of patients and controls

	Patients			Control		
	Men	Women	total	Men	Women	total
Number	62(46%)	73(54%)	135	99(66%)	51(34%)	150
Age	51.3 ± 19.4*	53.2 ± 17.5*	52.33 ± 18.3*	37.4 ± 14	43.1 ± 14.9	39.43 ± 14.51
Cystatin C (mg/l)	2.57 ± 1.27*	2.32 ± 1.21*	2.43 ± 1.24*	0.73 ± 0.14	0.80 ± 0.14	0.75 ± 0.14
Creatinine (mg/l)	7.10 ± 5.10*	6.51 ± 3.41*	6.78 ± 4.26*	1.38 ± 0.31	0.90 ± 0.25 #	1.22 ± 0.37
Urea (mmol/l)	20.46 ± 10.0*	18.92 ± 8.52	19.63 ± 9.22*	13.66 ± 4.56	15.00 ± 13.9	14.11 ± 8.89
Uric acid mg/dl	6.53 ± 1.81*	6.07 ± 1.79*	6.28 ± 1.81*	5.96 ± 1.75	4.89 ± 1.76 #	5.60 ± 1.82

*: significant difference between patients and control.
#: significant difference between males and females in the same group.

Table 2. Area under the ROC curve of the studied markers

		AUC	SE	p-value	95% C. I.	
Cystatin C (mg/ml)	Males (n = 62)	0.963	0.016	0.000	0.932	0.995
	Females (n =73)	0.920	0.027	0.000	0.868	0.972
	Total (n = 135)	0.946	0.015	0.000	0.918	0.975
Creatinine (mg/ml)	Males (n = 62)	0.863	0.034	0.000	0.797	0.930
	Females (n =73)	0.954	0.019	0.000	0.916	0.992
	Total (n = 135)	0.907	0.019	0.000	0.869	0.944
Urea (mmol/l)	Males (n = 62)	0.719	0.045	0.000	0.631	0.807
	Females (n =73)	0.740	0.046	0.000	0.649	0.830
	Total (n = 135)	0.727	0.031	0.000	0.666	0.787
Uric acid (mg/dl)	Males (n = 62)	0.589	0.049	0.058	0.493	0.685
	Females (n =73)	0.716	0.047	0.000	0.623	0.809
	Total (n = 135)	0.619	0.033	0.001	0.553	0.684

Table 3. Sensitivity, specificity positive predictive value, negative predictive value and likelihood ratios of the studied markers

		cutoff	**sensitivity**	**specificity**	**PPV**	**NPV**	**PLR**	**NLR**
Cystatin C	Males	1.11	88.71	100	100	93.40	∞	0.11
	Females	1.11	82.20	100	100	79.69	∞	0.18
	Total	1.11	85	100	100	88.24	∞	0.15
Creatinine	Males	2.20	69.35	100	100	83.90	∞	0.31
	Females	1.25	91.8	96	97.10	89.09	22.9	0.09
	Total	1.98	77	99.4	99.05	81.21	128.3	0.23
Cys + or Cr +	Males		98.4	100	100	99	∞	0.016
	Females		98.6	100	100	98	∞	0.014
	Total		98.5	100	100	98.7	∞	0.015
Urea	Males	17	61.3	83	70.37	77.57	3.61	0.47
	Females	14.05	73.97	69	77.14	64.81	2.39	0.38
	Total	17.55	57.8	85.7	78	69.19	4.04	0.49
Uric acid	Males	6.18	48.38	78	58.82	70.91	2.20	0.66
	Females	5.21	75.34	67	76.39	65.38	2.28	0.37
	Total	6.31	48	71.4	60.19	78.10	1.68	0.73

PPV: positive predictive value NPV: negative predictive value PLR: positive likelihood ratio NLR: negative likelihood ratio

Table 4. Correlation between cystatin C, creatinine, urea, and uric acid in the hemodialysis patients.

		Cys	Cr	urea	Uric
Males	Cys	1	0.133	0.046	0.056
	Cr		1	0.728**	0.335**
	Urea			1	0.385**
	uric				1
Females	Cys	1	0.056	0.089	0.118
	Cr		1	0.619**	0.170
	Urea			1	0.320**
	uric				1
Total	Cys	1	0.104	0.075	0.101
	Cr		1	0.680**	0.264**
	Urea			1	0.358**
	uric				1

**: significant at $p < 0.01$

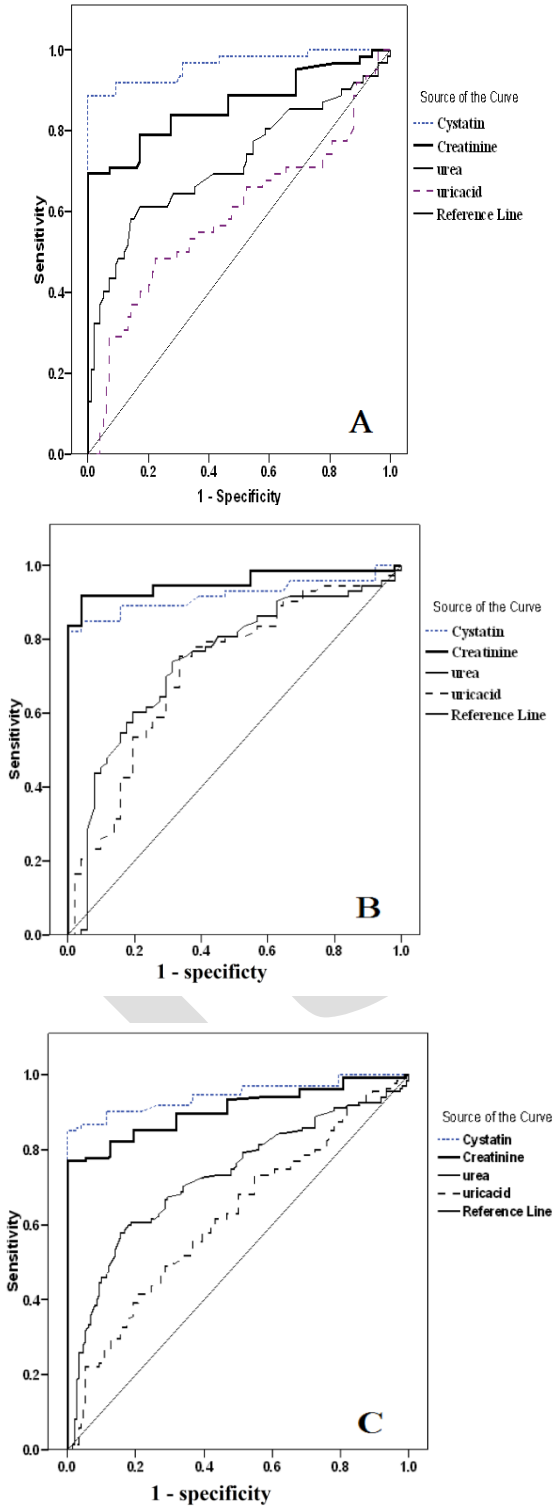

Figure 1. ROC curve for cystatin C, creatinine, urea and uric acid in males (A), females (B) and total (C).

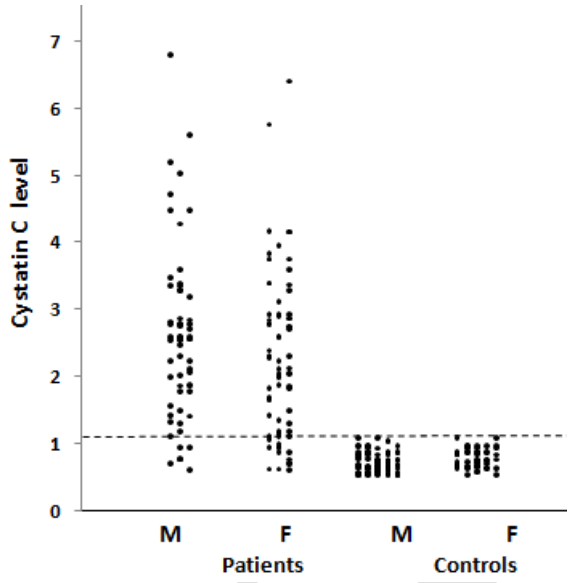

Figure 2. Scatter diagram showing the individual values of cystatin C in patients and control. The dashed line represents the optimum cut off value (1.11 mg/ml). The specificity is 100% and sensitivities are 88.71% and 82.2% for males and females, respectively.

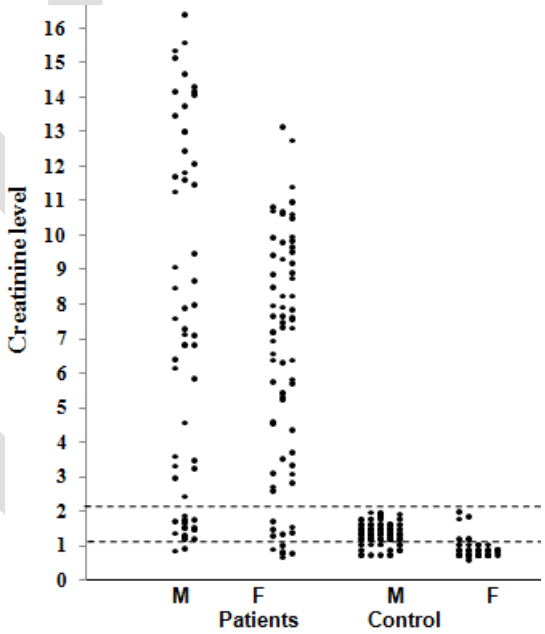

Figure 3. Scatter diagram showing the individual values of creatinine in patients and control. The dashed lines represent the optimum cut off values for males and females (2.20 mg/ml and 1.25 mg/ml, respectively). The control male values are under the line indicating 100% specificity whereas for females, the specificity was 96%. The sensitivities are 69.35% and 91.8% for males and females, respectively.

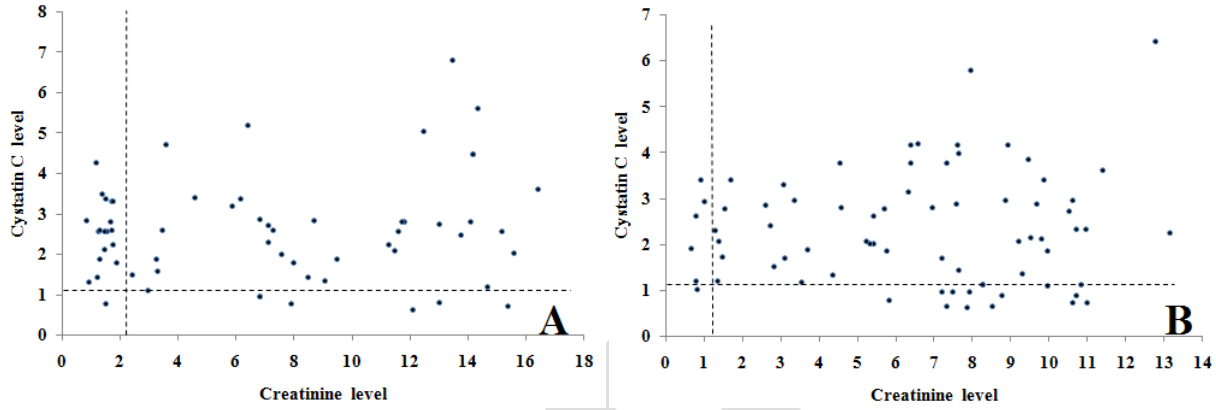

Figure 4. Scatter diagram showing the correlation between creatinine and cystatin C in the male patient group (A) and in the females (B). The dotted lines represent the optimal cut off values of creatinine and cystatin C.

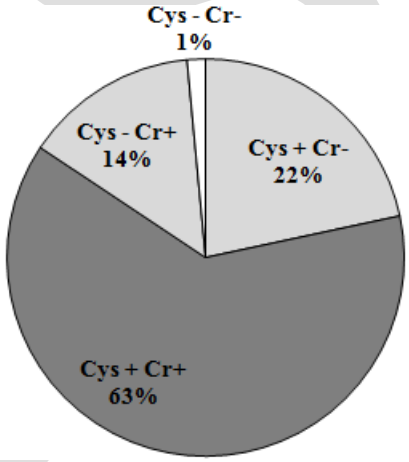

Figure 5. Sensitivity of cystatin C (85%) and creatinine (77%) solely and in combination (98.5%) in detecting early renal impairment.

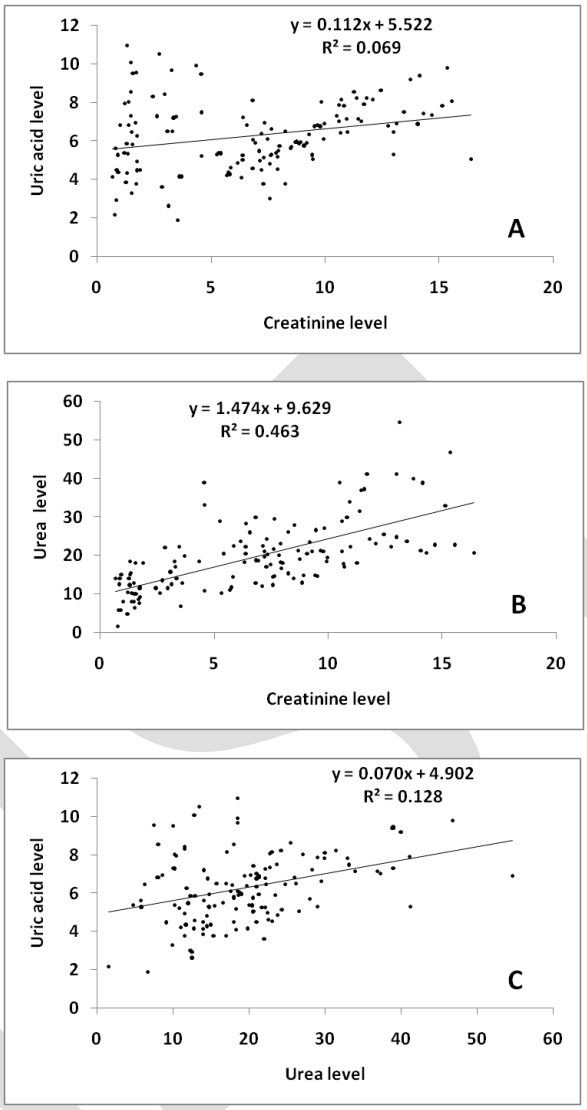

Fig. 6. Correlation between creatinine, urea, and uric acid in the patient group. A: correlation between creatinine and uric acid ($r = 0.264$, $p < 0.01$), B: creatinine and urea ($r = 0.680$, $p < 0.01$) and C: urea and uric acid ($r = 0.358$, $p < 0.01$).

Discussion

Early diagnosis of impaired renal function by using serum markers is extremely vital in saving life of patients. We therefore investigated the diagnostic value of serum cystatin C

concentrations in addition to creatinine as indicators of renal failure. Cystatin C has recently been introduced as an excellent marker of glomerular filtration rate that is not affected by many physiological and pathophysiological conditions (25). While patients with severely impaired renal function exhibit increased serum creatinine concentrations, detection of slightly or moderately decreased of glomerular filtration rate by widely used serum parameters is not possible, so the need for other serum markers such as cystatin C is urgently needed (26).

For clinical decision making, the selected cut-off value of a laboratory test should provide the best diagnostic accuracy for either ruling out or ruling in the particular disease. The ROC curve is a graphic method used to determine this optimal cut-off level (27, 28).

In the present investigation, we found that serum cystatin C, creatinine, urea and uric acid concentrations are significantly increased in patients with impaired renal function compared to the control group. Based on elevated serum levels of these parameters, we used the ROC curve analysis to evaluate the diagnostic value for the markers in detecting early renal impairment. ROC curve analysis supported an advantage of serum cystatin C over serum concentrations of creatinine, urea and uric acid. When the group of renal failure patients was considered as a whole, cystatin C (AUC = 0.946) was superior to creatinine (AUC=0.907, $p < 0.05$).

In the subgroup of female patients, we found that cystatin C (AUC = 0.920) was not superior to creatinine (AUC = 0.954, $p > 0.05$). The reverse was found in the male patient group where the AUC of cystatin C was 0.963 compared to 0.863 for creatinine, thus, creatinine was more sensitive than cystatin C in the females in contrast to male patients. Urea and uric acid were even less diagnostically efficient. At the optimal cut off values of simultaneous use of cystatin C and creatinine, the predictive values positive and negative were 98.7% and 100%, respectively. The positive and negative likelihood ratios were infinity (∞) and 0.015, respectively.

Among the most important probabilities related to diagnostic tests is the likelihood ratio. The positive likelihood ratio is the ratio of true positive rate and the false positive rate whereas the negative likelihood ratio is the ratio of false negative rate and the true negative rate. A positive likelihood ratio of 10 means that a positive response of the test is 10-fold more frequent among patients than the controls. A negative likelihood ratio equal to 0.10 means that a negative response of the test 10-fold less frequent among patients than the controls (29). In the present investigation infinite PLR obtained for simultaneous use of cystatin C and creatinine necessitates that any undiagnosed control subjects fulfilling the criteria (positive for either marker) will be 100% suffered from early renal impairment but still not diagnosed. The same may also be extended to the NLR where in simultaneous determination of both markers, the NLR was 0.015 meaning that a negative response of the test is 67-fold (1/0.015) less frequent among patients with renal failure than among the controls.

In agreement with the present investigation, serum cystatin C has also been reported to have a higher predictive value for early diagnosis of renal impairment (30, 31). The clear advantage of cystatin C compared to creatinine and urea in those patients may be due to the fact that cystatin C is not dependent on muscle mass, activity, or nutritional status.

The absence of correlation between cystatin C and creatinine came in the favor for the present investigation where each of the two markers acts as a complement of the other in increasing sensitivity for early detection of renal impairment.

Based upon the findings of this study, it may be concluded that serum cystatin C is a valuable marker for early detection of renal impairment, but was found to be much more valuable when analyzed with serum creatinine.

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