The role of serum cystatin C as an early predictor for the diagnosis of chronic kidney disease in Mosul City

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ABSTRACT

Background: Diagnostic marker to detect chronic kidney disease (CKD) at early stages is important as early intervention can slow the loss of kidney functions. Serum cystatin C (sCysC) is said to be a superior marker for CKD compared to serum creatinine (sCr) and other known markers in the near past period to detect the mild GFR reduction between 60 and 90 ml/min/1.73m^2.

Objective: To detect for detecting the role of cystatin C as an early predictor for the assessment of chronic kidney disease patients.

Methods: Blood and urine samples from 185 patients suffering from various stages of CKD or subjects under the risk of CKD were taken from Ibn-Sina teaching hospital during the period from 15th of March 2012 to 10th of sept 2013. Serum Urea (sUr), serum uric acid (sUA), serum creatinine (sCr), serum cystatin C (sCysC), 24 hour excreted urine protein and 24 hour excreted urine creatinine where analyzed then compared to corrected creatinine clearance for each patient.

Results: The results showed that serum CysC started to change from abnormal border line level at stage 2 to clearly abnormal level at stage three of CKD comparing to other assessments which used sCr, sUr, sUA. The results also indicated that the urinary 24h excreted protein was starting to be observed at stage 3 of CKD.

Conclusions: The present study indicated that sCysC have the potential benefit for early detection of CKD especially in those with high risk before appearance of the symptoms and before occurrence of the complications.

Key Words: Chronic Kidney Disease; creatinine; sCys C; Urinary protein; urea; uric acid.

الخلاصة

الخلفية : الدلالة التشخيصية للكشف عن مرض الكلى المزمن في المراحل المبكرة تعتبر مهمة لأنها تحد من فقدان الكلى لوظائفها. يعتقد ان قياس مستوى مصل الدم للسيستاتين سي من اسمى المؤشرات لمراض الكلى المزمن بالمقارنة مع قياس مستوى مصل الدم للكرياتنين وغيرها من المؤشرات المستخدمة مسبقا للكشف عن الانخفاض البسيط في معدل ترشيح الكبيبة الدموية الكلوية بين ٦٠ و ٩٠ مل\دقيقة٧٣،١م^2.

الهدف: التحري عن دور السيستاتين سي كعامل كشف مبكر لتقييم لمرض الكلى المزمن.

طرق العمل: تم اخذ نماذج دم و بول من مستشفى ابن سينا التعليمي للفترة من ١٥اذار ٢٠١٢الى ١٠ايلول ٢٠١٣، وشملت الدراسة ١٨٥مريض يعانون من مرض الكلى المزمن في مراحل مختلفة من المرض. تم قياس مستويات مصل الدم لليوريا وحامض اليوريك و الكرياتنين و السيستاتين سي. كما تم قياس كمية البروتين المطروح مع البول خلال ٢٤ساعة وقياس كمية الكرياتنين المطروح مع البول خلال ٢٤ساعة ثم بعد ذلك تم مقارنة كل من المقاسات من جهة مع مستوى تصفية الكرياتنين المصحح لكل مريض حسب مساحة الجسم السطحية من جهة اخرى.

النتائج: اظهرت النتائج بأن قياس مستوى مصل الدم للسيستاتين سي بدا بالتغير الى مستوى الحدود العليا الطبيعيه في المرحله الثانيه ثم اصبح بمستوى مرضي عند المرحلة الثالثة لمرضى الكلى المزمن بالمقارنة مع كل من قياسات

مستويات مصل الدم للكرياتنين و اليوريا وحامض اليوريك. كما بينت النتائج بان قياس كمية البروتين المطروح مع البول خلال ٢٤ساعة بدأ بالظهور في المرحله الثالثه من مرض الكلى المزمن.
الاستنتاجات : اثبتت هذه الدراسة بان قياس مستوى مصل الدم للسيستاتين سي ذو فائدة للتشخيص المبكر لمرض الكلى المزمن خصوصا للأشخاص المعرضين للإصابة بهذا المرض قبل ظهور اعراضه وقبل حصول مضاعفاته.

The recent considerable interest based on better discrimination for chronic kidney disease (CKD) severity which leads to early recognition of kidney dysfunctions well before changes in sCr occurred, as it has been evidenced that small or relative increases in sCr are associated with a concomitant increase in patients mortality even before the increase in sCr reaches to an abnormal limit[1, 2].

However, even serial evaluation of serum creatinine levels does not allow diagnosing adequately the rapidly evolving changes in renal function in severely ill subjects, therefore, attention has focused on the development of "early" biomarkers, enabling diagnosis of CKD long before creatinine levels start to increase. Logically, a combination of biomarkers could enhance diagnostic accuracy, but validation of current individual candidate biomarker is still underway[3,4,5].

The limitations of serum creatinine as a measure of glomerular filtration rate (GFR) have led to an extensive search for a more sensitive laboratory marker of impaired kidney filtration function[6].

Serum cystatin C level predicts mortality and morbidity more strongly than serum creatinine level in CKD and impaired kidney filtration affections[7]. Cystatin C is relatively stable, and can be rapidly, accurately, and precisely analyzed by automated analyzers, thereby meeting the practical requirements of a suitable laboratory test[8,9].

It is well known that serum creatinine is an insensitive measure of renal functions and only loosely corresponding to GFR. Moreover, serum creatinine is not an accurate reflection of GFR in the non-steady state, and is influenced by many factors, including muscle mass, gender, diet, liver functions, and age[10].

Serum cystatin C is well known clinically applicable biochemical parameter for assessment of acute kidney failure and it is also now a days start to be an interesting biochemical parameter in assessment of CKD patients (unfortunately not yet for our locality). Furthermore it is useful aid for staging of CKD as it can reflect more rapidly the early changes in kidney functions comparing to the traditional used parameters[11].

Urea clearance was one of the first clearance tests performed. Urea is freely filtered at the glomerulus and approximately 40% reabsorbed by the tubules. For this reason, it does not provide a full clearance assessment[12].

Serum uric acid is commonly elevated in subjects with chronic kidney disease (CKD), but was historically viewed as an issue of limited interest. Recently, uric acid has been reassessed as a potential contributory risk factor in the development and progression of CKD. Most studies documented that an elevated serum uric acid level independently predicts the development of CKD[13].

The aim of the current study was to detect the role of serum cystatin C as an early predictor for the assessment of

patients with chronic kidney disease in Mosul City.

Subjects, materials and methods

The patients included in this study were recruited from Ibn-Sina teaching hospital during the period from 15th of March 2012 to 10th of sept 2013.This study included 185 patients suffering from or under the risk of CKD. The studied samples consisted of 124 (67%) males and 61 (33%) females. These studied samples were classified according to the National Kidney Disease Education Program (NKDEP) Classification [14] depending on the corrected creatinine clearance into five stages of CKD.

1. Stage (1), included twenty five subjects under risks of Kidney damage with normal or elevated GFR GFR > 90 mL/min per 1.73 m2.
2. Stage (2), included forty seven subjects with Kidney damage and mild decrease in GFR GFR of 60 -89 mL/min per 1.73 m2.
3. Stage (3), included forty six patients with kidney damage and Moderate decrease in GFR GFR of 30 – 59 mL/min per 1.73 m2.
4. Stage (4), included twenty patients with kidney damage and severe decrease in GFR GFR of 16 – 29 mL/min per 1.73 m2.
5. Stage (5), included forty seven patients with kidney damage and Kidney failure - End Stage Renal Disease (ESRD) GFR of <15 mL/min per 1.73 m2.

Patients with the following criteria were excluded from the study:

A. Patients with thyroid diseases.
B. Patients with impaired renal function who are receiving corticosteroids.
C. Patients with recent attack of coaggulopathic conditions.
D. Patients on therapies affecting the measured parameters.
E. Pregnant and lactating women.
F. Patients suffered from any type of malignancy.

Venous blood sample (about 10 ml) was collected from each individual of the studied groups in a plain tube. The tube samples were then placed aside for 15-20 min to enable blood coagulation to occur. The tubes were then centrifuged for 10 min at 4000 RPM in order to obtain serum samples, separated and collected in aliquots and deep frozen at -20Cº until analyzed. Urine (24h) samples were also collected. Laboratory tests were achieved on serum and 24h urine samples.

Serum urea was measured by enzymatic method[15], using a kit supplied by Biomerieux company (France). The measurement of serum and urine creatinine were performed using a kit supplied by Randox company (U.K)[16]. Serum uric acid was determined quantitatively by enzymatic colorimetric method utilizing kit supplied by a Randox company (U.K)[17]. Serum cystatin C measurement ST AIA-PACK CysC is designed for the quantitative measurement of sCysC in human serum on TOSOH AIA System Analyzers. [18]. Urine Protein testing by Sulphosalisylic Acid[19].

Statistical analysis

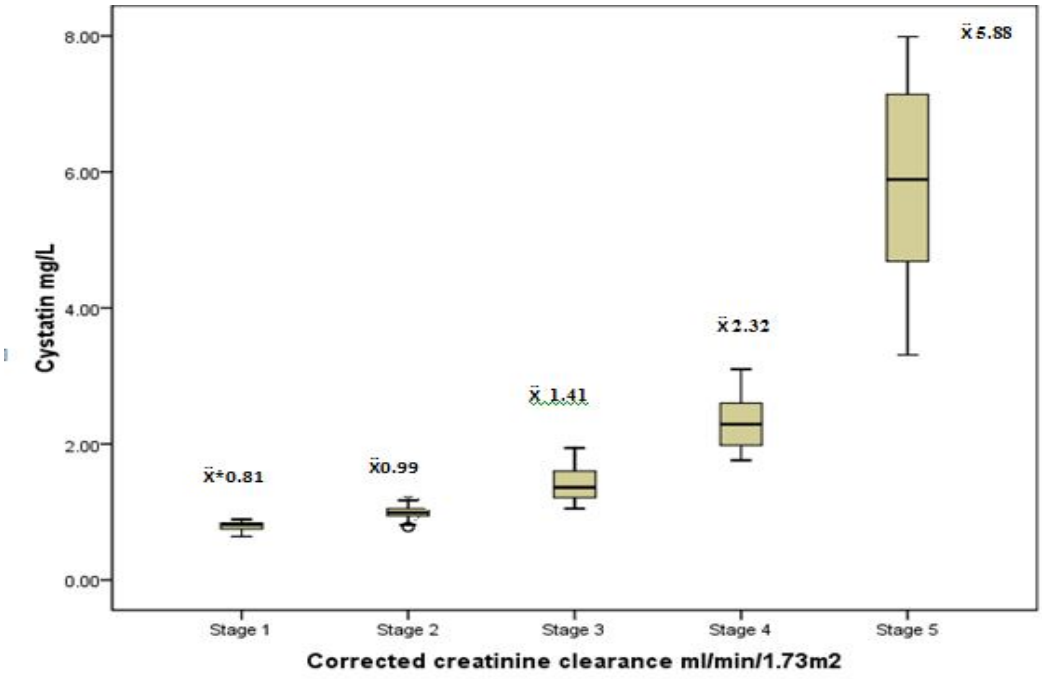
The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) program (version 17)[20].

Results

The results of this study showed that sCysC started to change for abnormal border line level at stage 2 and became clearly abnormal level at stage 3 CKD (fig. 1) comparing to other assessments which used sCr, sUr, sUA (fig. 2, 3, 4 respectively) which may be more useful

for early assessment of CKD. The sUA presented with positive relation to progression of CKD stages from 1-4 with mild decrease at stage 5 comparing with previous stage 4 (fig. 4). The results also showed that 24h excreted protein (fig. 5) is helpful biochemical parameter for early staging of CKD. In (fig. 6) we can see that despite the decrease of 24h urine creatinine excretion through the early stages of CKD which looks logically, in stage 5 there was an increase in the 24h creatinine excretion which reflect unexpected result.

The results table shows all parameters in mean ±SD against the five stages of CKD among the studied subjects.



X̄= mean

Figure 1. showing the change in sCysC in different stages of CKD

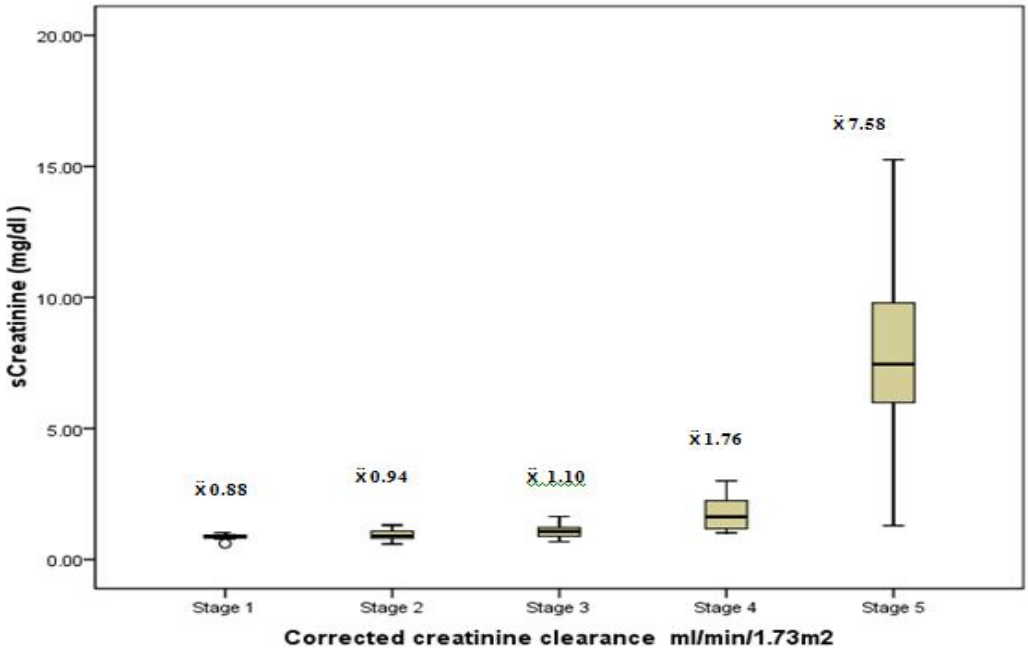


Figure 2. showing the change in sCr in different stages of CKD

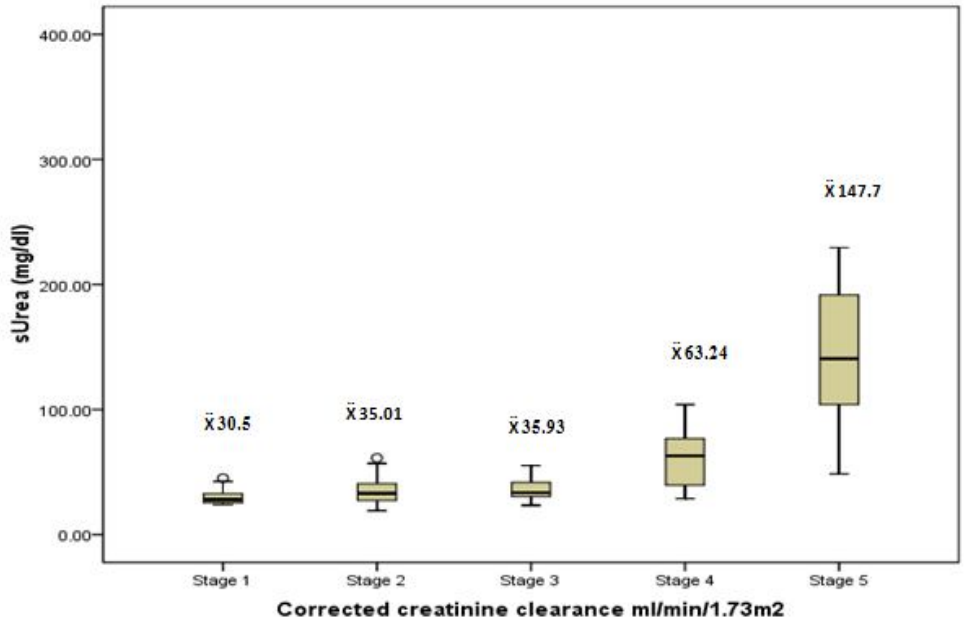


Figure 3. showing the change in sUr in different stages of CKD

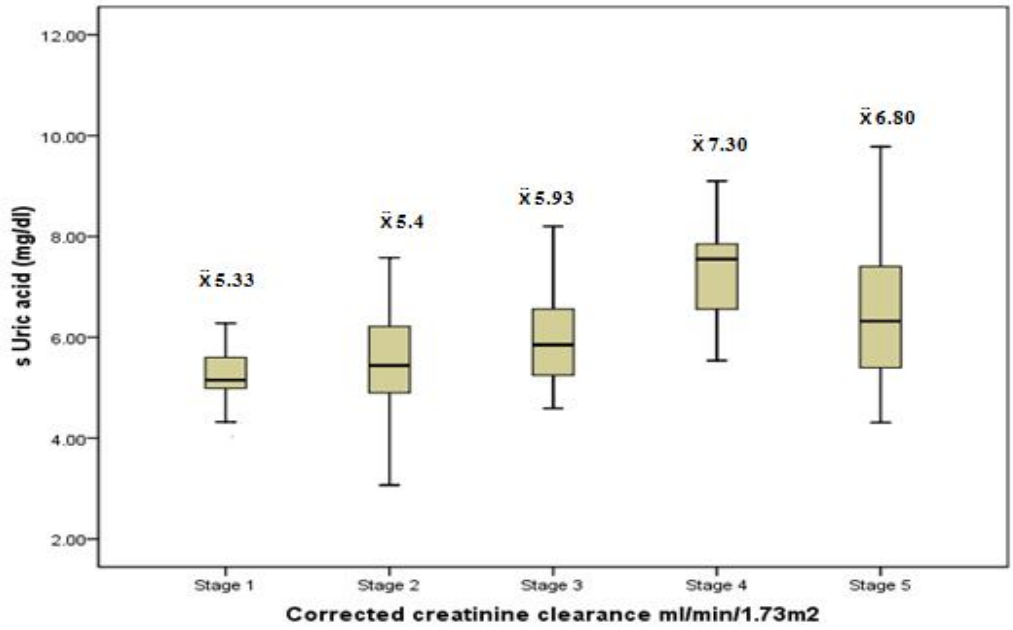


Figure 4. showing the change in sUA in different stages of CKD

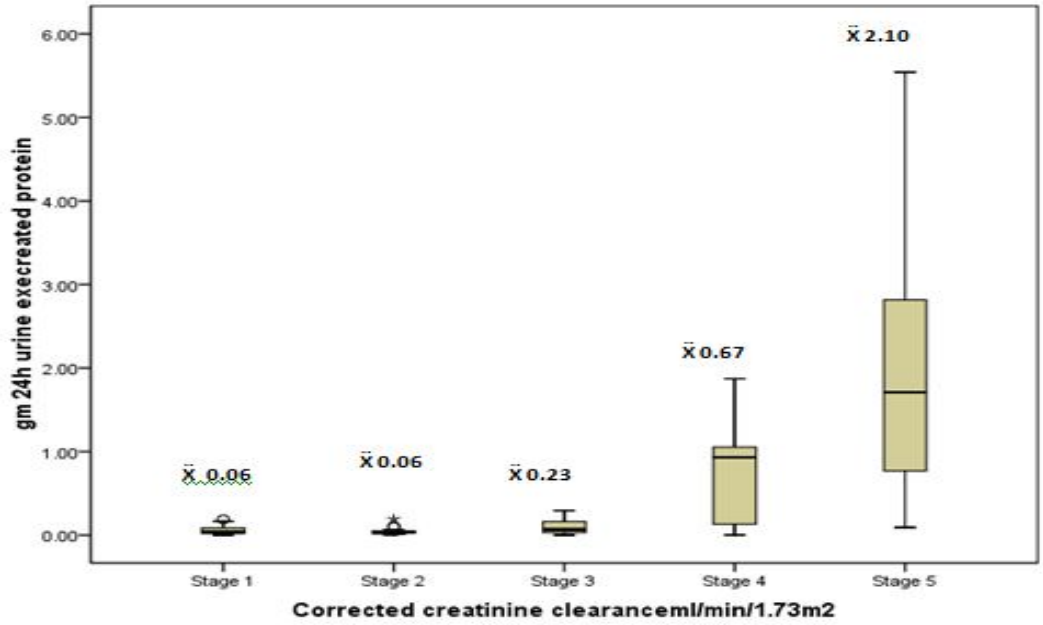


Figure 5. showing the change in urine excreted protein in different stages of CKD

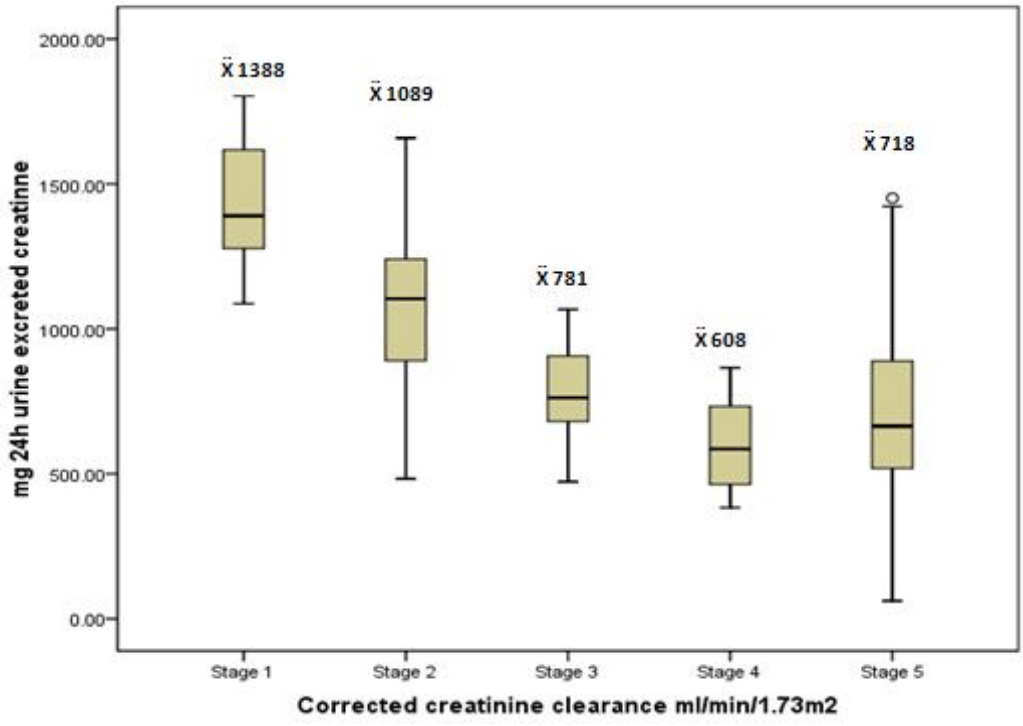


Figure 6. showing the change in urine excreted creatinine in different stages of CKD

Table 1. showing the mean ± standard deviation of all parameters against the five stages of CKD

parameter	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
sCysC mg/L	0.81±0.11	0.99±0.12	1.41±0.25	2.32±0.42	5.88±1.5
sCr mg/dl	0.88±0.11	0.94±0.20	1.1±0.27	1.76±0.64	7.58±3.58
sUr mg/dl	30.5±6.75	35.01±10.41	36.93±7.92	63.24±22.95	147.7±67.58
sUA mg/dl	5.33±0.86	5.4±1.15	5.93±0.81	7.3±1.03	6.8±1.95
gm 24 H excreted urine protein	0.06±0.06	0.06±0.09	0.23±0.52	0.67±0.6	2.1±1.61
mg 24 H excreted urine creatinine	1388±312.8	1089±307.5	781±172.2	608±156.9	718±344.6

Discussion:

Serum cystatin C has been proposed as an ideal marker for assessing the GFR. A number of studies have shown that sCysC is a more sensitive indicator of an early and mild reduction in renal functions than sCr[21, 22,23].

The results of the current study showed that sCysC started to change for abnormal border line level at stage 2 and became clearly abnormal level at stage 3

CKD comparing to other assessments which used sCr, sUr, sUA which may be more useful for early assessment of CKD. This result looks logically correct as sCysC level is stable and independent of body weight, height, and muscle mass. It is produced at a stable rate, which is unaffected by sex, age, diet, and nutritional status[9]. However, it is relatively recently introduced, there are only limited numbers of studies for sCysC in the Middle East general population.

Serum cystatin C results agree with a study accomplished in Malaysia which showed that sCysC increased with the progression CKD, and it was significantly higher in subjects with mild to moderate estimated GFR (eGFR) (stages 2 and 3.), but not significant in stage 1. This Malaysian study mentioned that sCysC has been demonstrated to be more accurate than serum creatinine in the detection of early renal impairment and in specific populations may allow for early detection of renal disease [24]. Astor et al 2012 concluded that sCysC had advantage over eGFR CKD in predicting kidney failure and it may be helpful in improving estimation of risk associated with decreased kidney function beyond current estimates based on eGFR CKD(Serum creatinine-based estimated glomerular filtration rate calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation)[25].

A Study conducted upon multi-Multi-Ethnic atherosclerosis affected patients mentioned that sCysC level and microalbuminuria are independent risk factors for incident CKD stage 3 and could be useful as screening tools to identify those at increased risk[26]. Whereas another study by katharina et al. reported that the diagnostic performance of serum creatinine, sCysC, or β-Trace Protein (BTP) for detecting even minor degrees of deterioration of renal function is very helpful[27].

However, unfortunately there is evidence that sCysC level may be changed independent to the renal function in case of inflammation, and like many other markers of inflammation, its plasma concentration may be higher in patients with decreased renal clearance[28]. Peralta et al reported that among adults diagnosed with CKD using the creatinine-based CKD-EPI equation, the adverse prognosis is limited to the subset who also have CKD according to the sCysC-based equation, so sCysC may have a role in identifying persons with CKD who have the highest risk for complications[29].

A recent study by Min et al in a meta-analysis demonstrated significant correlations between sCysC, SCr and GFR. Cystatin C was more sensitive, but less specific in early diagnosis of CKD, than SCr for the estimation of GFR [30]. Xun et al concluded that sCys C is more sensitive in diagnosis of early stages of renal impairment comparing to other conventional assessments methods[31]. The results of this study also show that 24h urinary excreted protein is helpful biochemical parameter for early staging of CKD but unfortunately the obstacles of 24h urine collection and possible errors by the patient during that collection limits its usage. Urinary albumin excretions were strongly correlated with the renal endpoint[32].

On the other hand despite the decrease of 24h urine creatinine excretion through the early stages of CKD which looks logically, in the stage 5 there was an increase in the 24h creatinine excretion which reflect unexpected result which could be as a result of strict supervision by the

managing doctors which may affect the potential of the present renal functions. Concerning the use of 24h corrected creatinine clearance, it like the assessment of 24h urinary protein excretion, the only limitation was that patients could have made errors during the 24-hour urine collection, which will negatively affect the predictive performance of the 24-hour urinary creatinine excretion otherwise it may be considered as the most actual indicator for measuring the early renal nephropathy[4].

Serum uric acid and serum urea showed significant increase in their concentration in late stage of CKD specially in stage 4 and 5 but not at early stages 1, 2 and 3, this may indicate that those two indicator are only good in late stages of CKD prediction with further limitation as those two parameters are non specific and may be increased in different other medical conditions like increased protein food intake, debilitating diseases, in acute systemic infections and malagnancy. This agrees with Diana et al results whom indicated that serum uric acid levels are a strong predictor of micro- or macro-albuminuria in patients with CKD with several limitations[33].

In another recent study accomplished by Meena et al, the study demonstrated a strong independent association between uric acid level and protein urea without association with decreased renal function[34] while in large, prospective cohort study of type 1 diabetic patients without proteinuria, Linda et al found that serum uric acid was an important predictor of the development of early GFR loss[35]. Giacomo et al. 2012 conclude that in type 2 diabetic individuals with preserved kidney function, hyper-uricemia seems to be an independent risk factor for the development of incident CKD[36]. While the present result shows mild decrease in stage 5 CKD comparing to stage 4 for sUA, this could be attributed to protein intake restriction advised by managing doctors.

Conclusions: The present study indicated that sCysC have the potential benefit for detecting early CKD especially in those with high risk.

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