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# Serum bilirubin: a simple routine surrogate marker of the progression of chronic kidney disease

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**ABSTRACT**

**Background:** Studies suggest that Chronic Kidney Disease (CKD) is a global burden health associated with significant comorbid conditions. Few biochemical parameters have gained significance in predicting the disease progression. The present work aimed to study the association of the simple biochemical parameter of serum bilirubin level with the estimated glomerular filtration rate (eGFR), and to assess their association with the co-morbid conditions in CKD.

**Methods:** We recruited 188 patients with CKD who attended a Nephrology out-patient department. eGFR values were calculated based on the serum creatinine levels using CKD-EPI formula. Various biochemical parameters including glucose, creatinine, uric acid, total and direct bilirubin were assayed in all study subjects. Study subjects were categorized into subgroups based on their eGFR values and their diabetic status and the parameters were compared among the different subgroups.

**Results:** We observed a significantly decreased serum bilirubin levels ($p < 0.001$) in patients with lower eGFR values, compared to those with higher eGFR levels. There was a significant positive correlation between the eGFR levels and the total bilirubin levels ($r = 0.92$). We also observed a significant positive correlation between the eGFR levels and the direct bilirubin levels ($r = 0.76$). On multivariate linear regression analysis, we found that total and direct bilirubin independently predict eGFR, after adjusting for potential confounders ($p < 0.001$).

**Conclusions:** Our results suggest that there is significant hypobilirubinemia in CKD, especially with increasing severity and co-existing diabetes mellitus. This finding has importance in the clinical setting, as assay of simple routine biochemical parameters such as serum bilirubin may help in predicting the early progression of CKD and more so in diabetic CKD.

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Bilirubin; estimated glomerular filtration rate; chronic kidney disease

## Introduction

Chronic kidney disease (CKD) is a global public health problem with increasing incidence worldwide.[1] The main diagnostic measure of CKD is glomerular filtration rate (GFR). Though the gold-standard method to measure GFR is by measuring inulin clearance, for practical purposes, we use estimated glomerular filtration rate (eGFR) calculated from serum creatinine employing different formulae.[2] Most patients with CKD progress into end stage renal disease or succumb to co-morbidities associated with progressive CKD such as diabetes mellitus and cardiovascular diseases.[1,2] Hence prediction of progression of CKD is of utmost importance to optimize their care plan. Early stages of CKD are asymptomatic and screening is necessary to detect CKD in its early stages. Detection of CKD is beneficial in even apparently normal subjects, so that early treatment can be instituted in them to prevent progression, to avoid further renal insults and to address cardiovascular risk factors.

At present, eGFR is the most valuable parameter for diagnosing CKD. However, eGFR is not measured routinely in apparently normal subjects. Equally pertinent is the fact that race and ethnicity are known to influence GFR.[2] The currently used eGFR equations, developed for the western population may not be suitable for Indians. Hence, the search for possible other routine parameters, to enable early identification of CKD and its progression gains importance.

Recent studies identify a potential reno-protective role for serum bilirubin probably due to its antioxidant, anti-inflammatory and cytoprotective effects.[3–6] Though some studies report it to be nephrotoxic, especially at very high levels, few studies have reported an association between serum bilirubin and eGFR (CKD).[4] Bilirubin has been reported as having anti-oxidant activity, and mildly elevated levels have been identified to be protective in many diseases such as coronary artery disease, peripheral vascular disease, stroke, non-alcoholic

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fatty liver, metabolic syndrome and cancer, where oxidative stress is a potential pathogenic factor.[3] CKD is a further example of a disease where oxidative stress is reputed to play crucial roles in initiation and progression of the disease.[5] Higher serum total bilirubin concentration is associated with lower risk of renal insufficiency.[6] Low bilirubin levels may provide a clue in early identification of CKD and its progression. Although some studies suggest a renoprotective role for bilirubin, others report that higher serum bilirubin levels may have a possible role in contributing to the development of renal failure, possibly linked to jaundice-related nephropathy.[7,8] Hence there is no clear-cut evidence that nephrotoxicity is associated with an increase or decrease in the bilirubin levels. Consequently, we hypothesise that serum bilirubin is associated with the eGFR, and in the present work we aim to study the correlation of bilirubin levels with eGFR in Indian adults. In doing so we consider the view that bilirubin levels may help in predicting the early progression of CKD and the associated co-morbid conditions in CKD.

## Materials & methods

This was a cross sectional study conducted in the departments of Biochemistry and Nephrology in a tertiary health care centre in Puducherry, South India. Ethical approval was obtained from the Institute's Human Ethics Committee (Approved as Project No. JIP/IEC/SC/2015/13/534 dated 20 April 2015). The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment in the study.

One hundred and eighty-eight patients attending a nephrology clinic in a tertiary care hospital in South India were recruited and staging of CKD was performed in accordance with the National Kidney Foundation practice guidelines for CKD.[9] eGFR was calculated in all study subjects using CKD-EPI formula using serum creatinine values, age, gender and race.[10] Exclusion criteria were age <18 years and >60 years (the upper limit of age is taken as 60 years as patients with more than 60 years of age are considered as elderly population), end stage renal disease (eGFR < 15 ml/min/1.73 $m^2$), patients with pre-existing heart disease, hepatic disease, hypertension, hematologic or hemolytic disease, malignancies, alcoholism, pregnancy, and use of drugs known to affect bilirubin levels such as theophylline, phenobarbitones, methotrexate, allopurinol and diuretics.

The clinical characteristics and detailed family history and presence of any co-morbid conditions were entered in a predetermined proforma. Gender, age, blood pressure, concomitant medications, and alcohol consumption were recorded in all study subjects. The subjects were further sub-grouped based on the eGFR values into different stages of CKD according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines [1] and their fasting glucose levels according to the American Diabetes Association guidelines [11] and the various parameters were compared between the sub-groups.

Following written informed consent, 5 ml blood was drawn from all participants after a fasting period of 12 h. Serum total bilirubin, direct bilirubin, fasting blood glucose, fasting lipid profile, serum aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase, serum uric acid, blood urea and serum creatinine levels were assayed by routine methods in fully automated clinical chemistry autoanalyzers.

### *Sample size estimation*

The sample size was estimated assuming the hypothesis of a conservative correlation of >0.3 between bilirubin and eGFR. To achieve this at $2p < 0.01$ and a 1-β power of 0.9, the sample size was calculated as 138. To gain better confidence in view of multiple measurement and to ensure enough power for the between groups analysis and multivariate linear regression analysis, this sample size was increased by a third, i.e. to 184. We finally recruited 188 study subjects in the present study.

### *Statistical analysis*

Both descriptive and inferential statistics were used. Baseline characteristics of the subjects are presented as descriptive statistics. Categorical data such as gender and clinical factors were described using percentages and frequencies and were compared between sub-groups by using Chi square test. The normality of continuous data was assessed by Kolmogrov–Smirnov test. The normally distributed continuous data such as total bilirubin, direct bilirubin, fasting blood glucose, fasting lipid profile, serum aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase, serum uric acid, blood urea and serum creatinine levels were described by mean with standard deviation whilst median and interquartile range were used for non-Gaussian data. Normally distributed continuous data were compared between sub-groups by ANOVA and Kruskal Wallis H test was used for non-Gaussian data. Correlation analysis were performed to study the association between bilirubin, uric acid and eGFR in the study subjects. $p < 0.05$ was considered as statistically significant.

## Results

The 188 patients were sub-divided, based on their eGFR values into CKD stages based on KDIGO guidelines [1]: 1 (>90 ml/min/1.73 $m^2$), 2 (60–89 ml/min/1.73 $m^2$), 3 (30–59 ml/min/1.73 $m^2$) and 4 (15–29 ml/min/1.73 $m^2$). Subjects with severe renal disease, i.e. stage 5 CKD [eGFR < 15 ml/min/1.73 $m^2$] were excluded from the study. Among the 188 subjects, 17.6% (33) belonged

**Table. 1.** Clinical, laboratory and demographic features of the subjects.

| Variable | Mean ± S.D. |
|---|---|
| Age (years) | 48 ± 9 |
| Gender, *N* (%) | |
| Males | 125 (66. 5) |
| Females | 63 (33.5) |
| Diabetes, *N* (%) | 70 (37.2 ) |
| Fasting blood sugar (mmol/L) | 6.5 ± 1.8 |
| Urea (mmol/L) | 22.4 ± 5.8 |
| Creatinine (micromol/L) | 145.5 (90.9–263.6) |
| Uric acid (micromol/L) | 359.6 ± 73.7 |
| Total protein (mg/L) | 72.2 ± 9.02 |
| Albumin (mg/L) | 39.5 ± 4.8 |
| Total bilirubin (micromol/L) | 13.2 ± 4.6 |
| Direct bilirubin (micromol/L) | 5.1 ± 1.9 |
| Aspartate aminotransferase (IU/L) | 22.6 ± 6.4 |
| Alkaline aminotransferase (IU/L) | 26.9 ± 6.5 |
| Alkaline phosphatase (IU/L) | 350.0 ±117.2 |
| Total cholesterol (mmol/L) | 7.2 ± 0.5 |
| Triglycerides ( mmol/L ) | 2.9 ± 0.4 |
| High density lipoprotein (mmol/L) | 0.89 ± 0.09 |
| Low density lipoprotein (mmol/L) | 4.9 ± 0.5 |
| Very low density lipoprotein (mmol/L) | 0.6 ±0.08 |
| Systolic blood pressure (mmHg) | 113 ± 12 |
| Diastolic blood pressure (mmHg) | 76 ± 7 |
| eGFR (ml/ min/1.73 $m^2$) | 45.0 (22.8–77.9) |

*Note.* eGFR: estimated glomerular filtration rate.

to stage 1, 16.5% (31) belonged to stage 2, 23.4% (44) belonged to stage 3 and 37.2% (70) belonged to stage 4 CKD. Clinical, laboratory and demographic characteristics of the patients are shown in Table 1.

The clinical and biochemical characteristics of subjects according to eGFR category are summarized in Table 2. Compared to patients with normal or near normal eGFR, patients with lower eGFR had lower levels of serum total and direct bilirubin. Compared to patients with normal or near normal eGFR, patients with lower eGFR had higher levels of fasting blood glucose and uric acid. The other biochemical parameters were comparable between the groups.

When the study subjects were sub-divided into two categories based on fasting blood sugar – diabetic (FBS ≥ 7 mmol/L) and non-diabetic (FBS < 7 mmol/L) (criteria of the American Diabetic Association),[11] diabetic patients had lower eGFR values, lower levels of total and direct bilirubin and higher levels of serum uric acid and creatinine, as compared with non-diabetic subjects (Table 3). Hence serum bilirubin levels were lower in CKD patients who had associated comorbid condition such as diabetes mellitus. The other biochemical parameters were comparable between the groups. Pearson correlations were performed to determine the degree of association between the different study parameters. Levels of total bilirubin and direct bilirubin correlated positively with the eGFR values ($r = 0.92$, $p < 0.0001$ and $r = 0.76$, $p < 0.0001$ respectively). Uric acid levels showed a significant negative correlation with the eGFR values ($r = -0.93$, $p < 0.0001$). Fasting blood sugar values correlated negatively with eGFR levels, total bilirubin and direct bilirubin levels ($r = -0.38$, $p < 0.0001$; $r = -0.39$, $p < 0.0001$; $r = -0.24$, $p = 0.001$ respectively) and positively with uric acid levels ($r = 0.39$, $p < 0.0001$). Bilirubin

**Table 2.** Comparison of baseline biochemical parameters among the study subjects, based on stage of CKD … as per the KDIGO guidelines (1).

| Parameters | >90 ml/ min/ 1.73 $m^2$ Stage 1 CKD (*N*=33) | 60–89 ml/min/ 1.73 $m^2$ Stage 2 CKD (*N*=41) | 30–59 ml/min/ 1.73 $m^2$ Stage 3 CKD (*N*=44) | 15–29 ml/min/ 1.73 $m^2$ Stage 4 CKD (*N*=70) | *p*-value |
|---|---|---|---|---|---|
| Age (years) | 35.3 ± 7.7 | 41 ± 50.1 | 52.5 ± 5.4 | 52.2 ± 6.0 | <0.001 |
| Gender, *N* (%) | | | | | 0.01* |
| Males, *N* (%) | 22(66.7) | 17(41.5) | 30(68.2) | 56(80) | |
| Females, *N* (%) | 11(33.3) | 24(58.5) | 14(31.8) | 14(20) | |
| Diabetes, *N* (%) | 0(0) | 3(7.3) | 20(45.5) | 47(67.1) | <0.0001* |
| Fasting blood sugar (mmol/L) | 5.4 ± 0.9 | 6.1 ± 1.1 | 6.4 ± 2.2 | 7.4 ± 1.8 | <0.0001 |
| Urea (mmol/L) | 22.1 ± 7.5 | 25.0 ± 7.2 | 20.8 ± 4.7 | 22.0 ± 4.0 | 0.06 |
| Creatinine(micromol/L) | 72.7(63.6–72.7) | 90.9(86.3–100) | 154.5(136.3–170.4) | 290.9( 243.1–327.3) | <0.0001** |
| Uric acid (micromol/L) | 247.4 ± 19.3 | 310.3 ± 10.6 | 364.8 ± 29.2 | 437.9 ± 15.2 | <0.0001 |
| Total protein (mg/L) | 75.1 ± 9.1 | 73.3 ± 11.2 | 70.1 ± 8.7 | 71.6 ± 7.4 | 0.082 |
| Albumin (mg/L) | 37.9 ± 5.3 | 39.9 ± 5.0 | 40.7 ± 4.4 | 39.2 ± 4.4 | 0.069 |
| Total bilirubin (micromol/L) | 20.5 ± 1.3 | 15.8 ± 1.6 | 12.7 ± 1.3 | 8.5 ± 1.5 | <0.0001 |
| Direct bilirubin (micromol/L) | 7.9 ± 1.0 | 6.0 ± 1.3 | 4.2 ± 0.9 | 3.9 ± 1.4 | <0.0001 |
| Aspartate aminotransferase (IU/L) | 23.0 ± 6.7 | 24.3 ± 8.5 | 22.9 ± 6.0 | 21.3 ± 4.9 | 0.118 |
| Alkaline aminotransferase (IU/L) | 26.5 ± 6.1 | 28.3 ± 7.5 | 24.6 ± 5.6 | 27.7 ± 6.2 | 0.058 |
| Alkaline phosphatase (IU/L) | 358.2 ± 77.9 | 382.8 ± 207.5 | 329.9 ± 73.4 | 339.5 ± 70.8 | 0.156 |
| Total cholesterol (mmol/L) | 7.2 ± 0.3 | 7.1 ± 0.9 | 7.2 ± 0.3 | 7.2 ± 0.3 | 0.559 |
| Triglycerides (mmol/L) | 2.9 ± 0.4 | 2.9 ± 0.5 | 3 ± 0.3 | 2.9 ± 0.4 | 0.734 |
| High density lipoprotein (mmol/L) | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.650 |
| Low density lipoprotein (mmol/L) | 4.9 ± 0.4 | 4.8 ± 0.9 | 5.0 ± 0.4 | 4.9 ± 0.4 | 0.754 |
| Very low density lipoprotein(mmol/L) | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.734 |
| Systolic blood pressure (mmHg) | 113 ± 11 | 112 ± 12 | 113 ± 12 | 113 ± 12 | 0.934 |
| Diastolic blood pressure (mmHg) | 76 ± 8 | 75 ± 6 | 76 ± 7 | 76 ± 6 | 0.763 |

*Notes.* eGFR: estimated glomerular filtration rate.
*chi square test. Data presented as mean and standard deviation. *P* value from ANOVA
**Kruskal Wallis H test for creatinine which is expressed as median (Interquartile range)

**Table 3.** Comparison of baseline biochemical parameters between study subjects, based on their FBS values.

| Variables | <7 mmol/L *N* = 118 | ≥7 mmol/L *N* = 70 | *p*-value |
|---|---|---|---|
| Age | 46.9 ± 10.3 | 52.2 ± 6.5 | <0.0001 |
| Gender, *N* (%) | | | 0.08* |
| Males | 73(61.9) | 52(74.3) | |
| Females | 45(38.1) | 18(25.7) | |
| eGFR (ml/min/1.73 m$^2$) [%] | | | <0.0001* |
| >90 | 33(100) | 0(0) | |
| 60–89 | 38(92.7) | 3(7.3) | |
| 30–59 | 24(54.5) | 20(45.5) | |
| 15–29 | 23(32.9) | 47(67.1) | |
| Urea (mmol/L) | 22.9 ± 6.5 | 21.5 ± 4.4 | 0.101 |
| Creatinine (micromol/L) | 100.0(81.8–165.9) | 254.5(163.6–318.1) | <0.0001** |
| Uric acid (micromol/L) | 328.3 ± 69.9 | 412.3 ± 43.9 | <0.0001 |
| Total Bilirubin (micromol/L) | 15.1 ± 4.3 | 9.8 ± 2.8 | <0.0001 |
| Direct Bilirubin (micromol/L) | 5.7 ± 2.01 | 4.2 ± 1.3 | <0.0001 |
| eGFR (ml/ min/1.73 m$^2$) | 68.1 ± 34.6 | 29.5 ± 14.5 | <0.0001 |

*Notes.* Data presented as mean and standard deviation or median and interquartile range.
*chi square test. *P* value from *t* test
**Mann–Whitney U test for creatinine which is expressed as median (Interquartile range).

**Table 4.** Univariate and Multivariate regression analysis to identify the predictors of eGFR in patients with CKD ($r^2 = 0.92$).

| Predictors | Unadjusted odds ratio | *P* value | 95% CI | Adjusted odds ratio | *P* value | 95% CI |
|---|---|---|---|---|---|---|
| Fasting blood sugar | −7.25 | <0.0001 | −9.78 to −4.71 | −0.016 | 0.349 | −1.25 to 0.63 |
| Uric acid | −0.43 | <0.0001 | −0.46 to −0.41 | −0.494 | <0.0001* | −0.29 to −0.17 |
| Total bilirubin | 6.82 | <0.0001 | 11.75 to 15.12 | 0.366 | <0.0001* | 1.87 to 3.57 |
| Direct bilirubin | 13.43 | <0.0001 | 6.39 to 7.24 | 0.132 | 0.001* | 1.15 to 3.54 |

*Notes.* Dependent variable – eGFR. *Prediction is significant.

levels were significantly associated with eGFR on univariate analysis (Table 4).

To adjust for the effects of confounders on study parameters, a multivariate linear regression analysis was performed with eGFR as the dependent variable (Table 4). Total and direct bilirubin levels showed significant positive association with eGFR and hence are independent predictors of eGFR, even after adjusting for confounders such as fasting blood sugar and uric acid. ($p < 0.001$; $r^2 = 0.90$).

## Discussion

The prevalence of CKD is on the rise worldwide. The gold-standard diagnostic measure of CKD is glomerular filtration rate (GFR). However, accurate determination of the GFR requires a measure of inulin clearance, which is not always practical. Hence, an estimated GFR (i.e. eGFR) has been developed, which call for factors such as age, sex and serum creatinine.[10] Most patients with CKD progress to end stage renal disease (requiring dialysis or transplantation) or succumb to co-morbidities associated with progressive CKD such as ischaemic heart disease and stroke. Hence prediction of progression of CKD is most important to improve the quality of life of these patients and optimize their care. However, eGFR is not computed routinely in apparently normal subjects and also the currently used eGFR equations, developed from western data [10] has not been validated in Indians. Hence search is on for possible other routine parameters, to enable early detection and progression of CKD gains importance.

The pathogenesis of CKD is multifactorial. Recent studies implicate the role of oxidative stress in the pathogenesis of CKD.[11] Studies show that bilirubin is recognized as an anti-oxidant. Its levels have been shown to correlate inversely with markers of oxidative stress, as well as positively with anti-oxidant enzyme levels in CKD.[12] Serum total bilirubin concentration is associated with kidney disease progression in patients with CKD.[13,14] Bilirubin levels have also been shown to be protective against atherosclerosis, a disease that is intimately related to oxidative injury and with CKD.[15] Bilirubin inhibits TNF-α-related upregulation of endothelial adhesion molecules and also has anti-complement properties that protect against inflammation and development of atherosclerosis. It also has cytoprotective properties through its influence on protein kinase C. Hence, through these mechanisms, bilirubin could protect against progression of CKD. Hypobilirubinemia might be a possible risk factor of end stage kidney disease.[16] Hence we studied the association of bilirubin with eGFR in patients with CKD.

In this study, when we categorized the subjects based on their eGFR levels, we found that compared to patients with higher eGFR, patients with lower eGFR showed a decline in serum total and direct bilirubin. We found that on multivariate linear regression analysis, total and direct bilirubin concentrations showed a positive association with eGFR, even after adjusting for other confounders. Hence there is a state of hypobilirubinemia, as the severity of CKD increases (and eGFR value decreases), which may lead to uncontrolled oxidative stress and perhaps inflammation.[12] This is due to alleviation of its cytoprotective

properties against reactive oxygen species, inflammation and accelerated atherosclerosis, causing early progression of the disease, thus furthering the renal damage in CKD. The detection of lowered bilirubin levels may help in detecting the progression of CKD, so that appropriate timely intervention can be instituted. Thus a mildly elevated total and direct bilirubin values in the serum may be protective in patients with CKD. However, this finding requires validation in further longitudinal studies to assess the oxidative stress and inflammation and atherosclerosis in CKD and to correlate it with the bilirubin levels.

We also categorized the study subjects based on the co-morbid condition diabetes mellitus. We found that eGFR values were slightly lower in diabetics, compared to the non-diabetics, indicating that the co-existence of diabetes further worsened the CKD pathology, i.e. diabetic nephropathy.[17,18] Shin et al. [19] studied serum total bilirubin concentration and kidney function in Korean diabetic and non-diabetic adults demonstrated that serum total bilirubin concentration showed a linear trend with the eGFR values. Another study showed a positive correlation between serum bilirubin concentration and eGFR and showed that indirect bilirubin concentration was an independent determinant of eGFR and log of urine albumin excretion in patients with type 1 diabetes. [20] Higher bilirubin levels within the normal range were associated with significantly decreased risk of progression from microalbuminuria to macroalbuminuria and thus might be predictive of a lower risk of progression of nephropathy in type 2 diabetic patients.[17,18]

Patients with diabetes, characterized by a metabolic state of dysregulation between pro- and anti-oxidants, showed lower bilirubin levels. As the bilirubin levels decrease, there is increasing co-morbidity in diabetic CKD, due to unopposed oxidative stress and lowered anti-oxidant status, leading to increased risk and further progression of the disease process.[21] Hence there is a link between hypobilirubinemia and adverse cardiovascular outcomes in CKD. Hence, serum bilirubin concentration may be used as a provisional new risk factor for diabetic CKD.

We also observed a significant elevation in the levels of uric acid as the eGFR decreases and also a negative correlation between the two parameters. However, it is well known that uric acid increases in renal failure, as there is a deterioration in the renal function. Hence it is difficult to establish that uric acid is linked to eGFR. Nonetheless, uric acid was found to be significantly associated with eGFR and thus could independently predict the eGFR in CKD subjects. Thus we speculate that a mildly elevated total and direct bilirubin values may be reno-protective in the pathogenesis of CKD, as well as in its co-morbid conditions. Whether the lowered bilirubin levels in CKD is a cause or a consequence of the disease need to be explored in future studies. We note a number of limitation, such as that the study focusses on the middle-aged, having excluded those aged over 60 and those with severe renal failure (CKD stage 5), and the potential effects of prescribed drugs.

In conclusion, our results suggest that in middle-aged Indians there is significant hypobilirubinemia in CKD, especially with increasing severity and co-existing diabetes mellitus. Thus, a mild elevation in serum bilirubin might be renoprotective in CKD, a finding which requires further exploration in future studies. Our work is a progress in biomedical science because simple routine biochemical parameters like serum bilirubin, that can be measured easily in the laboratory and applied in medical practice, may help in predicting the early progression of CKD and may gain relevance in the out-patient setting.

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## Disclosure statement

The authors report no conflict of interest.

## References

[1] Levey AS, Atkins R, Coresh J, et al. Chronic kidney disease as a global public health problem: approaches and initiatives – a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007;72:247–259.
[2] Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. Am. J. Kidney Dis. 2014;63:820–834.
[3] Ryu S, Chang Y, Zhang Y, et al. Higher serum direct bilirubin levels were associated with a lower risk of incident chronic kidney disease in middle aged Korean men. PLoS ONE. 2014;9:e75178.
[4] Tanaka M, Fukui M, Okada H, et al. Low serum bilirubin concentration is a predictor of chronic kidney disease. Atherosclerosis. 2014;234:421–425.
[5] Kao MPC, Ang DSC, Pall A, et al. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J. Hum. Hypertens. 2010;24:1–8.
[6] Lee AT, Wang YY, Lin SY, et al. Higher serum total bilirubin concentration is associated with lower risk of renal insufficiency in an adult population. Int. J. Clin. Exp. Med. 2015; 8:19212–19222.
[7] Mairiang P, Bhudhisawasdi V, Borirakchanyavat V, et al. Acute renal failure in obstructive jaundice in cholangiocarcinoma. Arch. Intern. Med. 1990;150:2357–2360.
[8] Betjes MG, Bajema I. The pathology of jaundice-related renal insufficiency: cholemic nephrosis revisited. J. Nephrol. 2006;19:229–233.
[9] Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann. Intern. Med. 2003; 139: 137-147. Erratum. In: Ann. Intern. Med. 2003;139:605.
[10] Levey AS, Stevens LA, Schmid CH, et al. A new equation

to estimate glomerular filtration rate. Ann. Intern. Med. 2009;150:604–612.
[11] American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2016;39:S13–S22. doi:http://dx.doi.org/10.2337/dc16-S005.
[12] Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235:1043–1046.
[13] Kumar A, Pant P, Basu S, et al. Oxidative stress in neonatal hyperbilirubinemia. J. Trop. Pediatr. 2007;53:69–71.
[14] Sakoh T, Nakayama M, Tanaka S, et al. Association of serum total bilirubin with renal outcome in Japanese patients with stages 3–5 chronic kidney disease. Metabolism. 2015;64:1096–1102.
[15] Kawamoto R, Ninomiya D, Hasegawa Y, et al. Association between serum bilirubin and estimated glomerular filtration rate among elderly persons. PLoS ONE. 2014;9:e115294.
[16] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin. Chem. 1994;40:18–23.
[17] Ni shimura T, Tanaka M, Sekioka R, et al Serum bilirubin concentration is associated with eGFR and urinary albumin excretion in patients with type 1 diabetes mellitus. J. Diabetes Complications. 2015;29: 1223–1227.
[18] Toya K, Babazono T, Hanai K, et al. Association of serum bilirubin levels with development and progression of albuminuria, and decline in estimated glomerular filtration rate in patients with type 2 diabetes mellitus. J. Diabetes Investig. 2014;5:228–235.
[19] Shin HS, Jung YS, Rim H. Relationship of serum bilirubin concentration to kidney function and 24-hour urine protein in Korean adults. BMC Nephrology. 2011;12:29.
[20] Oda E, Aoyagi R, Aizawa Y. Hypobilirubinemia might be a possible risk factor of end-stage kidney disease independently of estimated glomerular filtration rate. Blood Press Res. 2012;36:47–54.
[21] Mashitani T, Hayashino Y, Okamura S, et al. Correlations between serum bilirubin levels and diabetic nephropathy progression among Japanese type 2 diabetic patients: a prospective cohort study (Diabetes Distress and Care Registry at Tenri [DDCRT 5]). Diabetes Care. 2014;37:252–258.

Summary table.

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| What is known about this subject |
| Glomerular filtration rate (GFR) is the main diagnostic measure of chronic kidney disease (CKD) which is a global health problem |
| CKD is associated with comorbid conditions like diabetes mellitus |
| Bilirubin levels are altered (increased or decreased) in CKD patients |
| What this paper adds |
| There is a mild reduction in the bilirubin levels (total and direct) in Indian adults with CKD, more so in the presence of co-morbidities |
| Bilirubin levels showed independent association with eGFR, even after adjusting for confounders |
| Simple routine laboratory tests like serum bilirubin can be useful in detecting the progression of CKD |