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Original Research Article

Association of Inflammatory Marker C-Reactive Protein and Interleukin-6 with Stages 3-5 of Chronic Kidney Disease

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Abstract

***Background*:** Despite different strategy to retard the progression of CKD, majority still progress to ESRD. Other than conventional risk factors inflammation is considered as one of the reversible factors responsible for CKD progression which can be intervened. Therefore, we have studied the level of inflammatory marker CRP and IL-6 at stages 3-5 of CKD patients and their association with CKD stage progression determined by eGFR. ***Methods*:** We have conducted a cross sectional study among 150 CKD patients and 32 healthy controls at OPD of Nephrology department of National Institute of Kidney Diseases and Urology, Dhaka from January`2016 to December`2016. Purposive sampling method was the tool for case selection and CKD was defined as eGFR <60ml/min/1.73m^2. AKI, history of dialysis, kidney transplant, patients having acute infection, malignancy or liver disease, received chemotherapy or immunotherapy (<6 months) and patients with connective tissue disease were the exclusion criteria. Fasting blood glucose, Hb%, serum Ca^{++}, serum PO_4, serum total cholesterol and serum triglyceride level were measured for both case and controls. eGFR has been calculated using MDRD equation. For the cases CRP had been measured on two occasions at an interval of one and half month. Those cases who had raised CRP level in both occasions were selected for measurement of IL-6 from second sample. CRP had been measured by turbidimetry method using fully automated chemistry analyzer. IL-6 had been measured using enzyme linked immunosorbent assay (ELISA) based on the Biotin double antibody sandwich technology. Scatter diagram and multiple regression models were used to examine the association between CRP and IL-6 with stage 3-5 of CKD denoted by eGFR. ***Results*:** The mean of CRP were 9.5±2.7 mg/L in CKD patients vs. 2.9±0.5 mg/L in controls without CKD and the mean of IL-6 were 29.5±13.0 pg/ml in CKD patients vs. 3.3±0.3 pg/ml in controls without CKD. Both were elevated and significant statistically (p=.001). Scatter diagram showing correlation coefficient (r value) -0.592 and -0.615 for eGFR vs. CRP and eGFR vs. IL-6 respectively which indicate both are negatively correlated with eGFR. But IL-6 had a more strong negative correlation. Multiple regression analysis shows regression co efficient (B) for CRP and IL-6 is -1.251 and -2.826 respectively after taking into account other factors which can alter eGFR level (age, Hb%, serum Calcium, S. Total Cholesterol, S.PO_4, S. Triglyceride) that indicates in the prediction of eGFR, IL-6 had significant negative association than CRP after adjusting the conventional factors responsible for CKD progression. ***Conclusion*:** Our data suggest that though CRP and IL-6 both are significantly raised in advanced CKD stage 3-5, IL-6 is more rapidly increasing and more strongly associated than CRP with the severity of CKD measured by eGFR.

Keywords: Chronic Kidney Disease, estimated glomerular filtration rate, C- Reactive Protein, Interleukin-6, inflammation and end stage renal disease.

INTRODUCTION

Chronic kidney disease (CKD) is highly prevalent worldwide and a major risk factor for end-stage renal disease (ESRD), cardiovascular disease (CVD), and premature death [1]. Several animal and human studies have shown that once renal insufficiency is established, its progression is inexorable, regardless of etiology. However, some nephropathies, such as diabetic nephropathy and polycystic kidney disease, have been reported to progress more rapidly [2] Similarly, patients whose kidney histology shows a higher degree of interstitial fibrosis also progress more rapidly to ESRD [3]. This has prompted the search for new risk factors/markers, recently resulting in research of a group of biochemical markers involved in several pathophysiological mechanisms of renal disease, such as inflammation [C-reactive protein (CRP) and interleukin-6 (IL-6) and its soluble receptor (IL-6R)], endothelial dysfunction (albuminuria, von Willebrand factor), dysregulation of the coagulation and fibrinolysis system (plasminogen activator inhibitor-1 and tissue-type plasminogen activator), and others (soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) [3-5].

Recent studies, both epidemiologic and basic science, have suggested that in the general population, chronic inflammation may have a stronger causal role in endangering atherosclerotic cardiovascular disease than LDL hypercholesterolemia [5]. This notion may lead to a major shift away from the traditional Framingham paradigm and toward the nontraditional paradigm of inflammation [6]. Inflammation seems to be at least one of the reasons for the high burden of atherosclerotic cardiovascular disease and death in individuals with CKD [7]. Patients with CKD and higher serum levels of inflammatory markers such as C-reactive protein (CRP) and IL-6 have a higher rate of CKD progression and poor clinical outcomes, including higher death [7]. And inflammation may be the missing link between the surrogates of malnutrition-wasting syndrome such as hypoalbuminemia and poor survival in patients with CKD, especially those who undergo maintenance dialysis treatment [8]. On the basis of these premises, many nephrologists are interested in relevant information about the inflammatory markers and their associations with both CKD progression and cardiovascular disease and death in this population.

OBJECTIVE

To investigate the level of inflammatory marker CRP and IL-6 with CKD patients belonging to stage 3 to 5.

METHODOLOGY

Study Design: Cross sectional study.

Place of Study: National Institute of Kidney Diseases and Urology. Sher-E- Bangla Nagar, Dhaka.

Duration of Study: From January 2016 to December 2016.

Study Population: Patients of CKD stage 3 to 5 aged 18 years and above.

Sample size:

$n= z^2pq/e^2$

Z=1.96

p=.86

(one study shows 86% of all ESRD patients have at least one elevated inflammatory biomarker) (Zoccali *et al.*, 2006)

q=1-p= .14

e= .05

So, n = 185

Sampling Method: Purposive sampling method.

Selection Criteria

For Case:

Inclusion Criteria:

1. Patients aged 18 years and above.
2. Diagnosed patients of CKD stage 3 to 5.
3. Patients who gave written consent.

Exclusion Criteria:

1. Patients suffering from acute kidney injury.
2. Patients had a history of kidney transplant and on dialysis.
3. Patients having acute infection, malignancy or liver disease.
4. Patients receive immunotherapy or chemotherapy in recent period (<6 months).
5. Patients having history of connective tissue disease.

Data collection tools and techniques:

1. Data collection sheet (Appendix I).
2. Clinical examination and
3. Blood sample were the tools and technique for data collection.

List of Variables:

The following variables were studied:

1. Demographic variables were Age, Gender, Residence, Education, Profession, Weight, and Height of patient.
2. Clinical variables related to CKD were DM, HTN, Glomerulonephritis, Obstructive uropathy, Polycystic Kidney Disease.
3. Laboratory variables were
   Haematological: Total WBC count, ESR, Hb%
   Biochemical: Serum creatinine, Serum total cholesterol and triglyceride, fasting blood sugar, serum calcium, serum phosphate, C Reactive Protein (CRP), urine albumin.
   Immunological: Serum interleukin-6(IL-6)

Study Procedures

This study was performed in two steps. During the first step patients were selected according to inclusion and exclusion criteria. Then relevant history, physical examination, and laboratory reports were recorded in the data collection sheet (Appendix I). Height, weight and blood pressure were measured. BMI was calculated from formula, BMI= weight in Kg/ height in meter^2. Blood samples were collected from the selected patients and allowed to clot for half an hour and then the samples were centrifuged for 15 minutes and serum was stored in ultra-deep freezer until analysis. These samples were marked as "sample A". After collection of sample, selected patients have been given a follow up date after one and half months and this was the second step.

At the second step blood samples were collected from the participants after an overnight fasting of at least 8 hours and again serum were separated and stored in ultra-deep freezer until analysis. These samples were marked as "sample B".

At the same time fasting blood samples were collected from the healthy persons and serum were separated and stored in ultra-deep freezer until analysis. These samples were marked as "sample C" and used as control.

CRP has been measured from both "sample A" and "sample B". Those patients who have raised CRP (> 5mg/L) in both the samples were included in the study and selected to measure IL-6.

IL-6, total cholesterol with triglyceride and fasting blood sugar level have been measured from sample B serum of selected patients. CRP, IL-6 and other laboratory parameters have also been measured from "sample C" of control group.

METHOD

Using enzyme linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology.

Assay Range:
02 ng/l(pg/ml) – 640 ng/l (pg/ml)
Sensitivity: 1.03ng/l(pg/ml)

Estimation of Serum CRP: (Appendix III) Serum CRP had been measured using Mindray C- Reactive Protein kit (188 test kit).

Method: Turbidimetry method using fully automated chemistry analyzer Mindray-BS-230.

Reference Intervals: <5.0 mg/L

Estimation of Serum Creatinine: (Appendix IV)

Serum Creatinine had been measured using Erba Mannheim Creatinine kit. Catalog No. XSYS0024, XSYS0076

Principle

Creatinine reacts with alkaline picrate to produce a reddish colour (Jaffe reaction). The absorbance of this complex is proportional to the creatinine concentration in the sample.

Method: Turbidimetry method using fully automated chemistry analyzer Erba-XL-200.

Estimation of eGFR: By using MDRD formula (National kidney disease education program, 2013)

Estimation of serum Cholesterol: (Appendix V)

Serum Cholesterol had been measured using Erba Mannheim cholesterol kit. Catalog No. XSYS0009, XSYS0070

Method: Turbidimetry method using fully automated chemistry analyzer Erba-XL-200.

Estimation of serum Triglyceride: (Appendix VI)

Serum Triglyceride had been measured using Erba Mannheim Triglyceride kit. Catalog No. XSYS0041, XSYS0071

Method: Turbidimetry method using fully automated chemistry analyzer Erba-XL-200.

Estimation of serum Calcium and Phosphate: Turbidimetry method using fully automated chemistry analyzer Erba-XL-200.

Facilities

Most of the investigations were carried out in the laboratory of the department of Biochemistry at National Institute of kidney Diseases and Urology, Sher E Bangla Nagar, Dhaka. CRP was investigated in a private biochemistry laboratory under supervision of a clinical Biochemist and IL-6 was being measured in an immunology lab.

Statistical Analysis:

The data was analyzed with the statistical software SPSS 21 for Microsoft Windows (SPSS Inc., Chicago, IL, USA). Quantitative variables were expressed as mean and standard deviation (±SD). Qualitative variables were expressed as the frequency of distribution of each category and percentage. Unpaired t-test and chi square test were used to compare between two groups. One way analysis of variance (one way ANOVA) was used to compare quantitative difference between multiple groups. Association was seen by Pearson correlation test and also by multiple regression model. Statistical significance was set at $P < 0.05$.

RESULT

Table I shows comparison of the clinical and laboratory parameters between case and control. It was observed that, the mean age and BMI were statistically not significant (p>0.05) between two groups that is case and control were matched according to age and BMI level. But eGFR and other laboratory parameters are statistically significant (p<0.05) between two groups that indicates there is significant difference in case and control on the ground of eGFR and laboratory parameters.

Table I: Comparison of the clinical and laboratory parameters between case (n=150) and control (n=32)

Variable	Case (n=150)	Control (n=32)	P value
	Mean±SD	Mean±SD	
Age in years	53.2±11.3	54.3±8.6	0.272
BMI (kg/m^2)	24.8±15.6	24.0±1.4	0.214
eGFR (ml/min/1.73 m^2)	25.9±12.5	103.0±8.9	0.001
Serum creatinine (mg/dl)	3.3±2.7	0.9±0.1	0.001
ESR (mm in 1st hour)	26±7	11±2	0.001
WBC (×10^9/L)	8.4±1.2	7.4±1.3	0.001
CRP (mg/L)	9.5±2.7	2.9±0.5	0.001
IL-6 (pg/ml)	29.5±13.0	3.3±0.3	0.001
Hb% (gm/dl)	11.4±1.4	13.5±0.7	0.001
Serum calcium (mg/dl)	8.3±0.5	8.9±0.4	0.001
Serum phosphate (mg/dl)	4.7±0.6	3.6±0.6	0.001
Total Cholesterol (mg/dl)	184.8±41.7	116.1±47.5	0.001
Serum triglycerides (mg/dl)	179.1±61.9	119.8±16.7	0.001
Serum FBS (mmol/L)	8.3±3.4	5.7±0.6	0.001

P value reached from unpaired t-test

Table II shows distribution of the cases and their mean value of laboratory parameters according to stages of CKD, it was observed that the highest number of cases are in stage 3 (n=55) which gradually declining to stage 5. The difference of age and BMI at different stages is not statistically significant but the differences in the mean value of laboratory parameters (serum creatinine, eGFR, Hb%, serum Ca^{++}, serum PO_4, serum total cholesterol, serum triglyceride, CRP and IL-6 level) of cases in each stage is statistically significant that indicate they are significantly related with the progression of stages of CKD.

Table II: Distribution of the cases (n=150) and their mean value of laboratory parameters according to stages of CKD

	CKD stage			P value
	Stage 3	Stage 4	Stage 5	
Number of cases	**55**	**50**	**45**	
	Mean±SD	Mean±SD	Mean±SD	
Age in years	53.7±10.2	53.7±13.0	54.4±12.0	0.945
BMI (kg/m^2)	23.3±3.4	24.2±4.5	23.5±3.2	0.445
Serum creatinine (mg/dl)	2.01±0.31	3.42±0.73	5.66±0.85	0.001
eGFR (ml/min/1.73 m^2)	40.21±5.92	22.54±4.3	12.02±.98	0.001
Hb% (gm/dl)	11.8±1.2	10.8±1.3	10.4±1.6	0.001
Serum Ca^{++} (mg/dl)	8.5±0.5	8.1±0.6	7.9±0.4	0.001
Serum PO^4 (mg/dl)	4.9±0.5	5.1±0.6	5.5±0.6	0.001
Serum Total cholesterol (mg/dl)	175.5±47.9	184.4±.34.4	195.7±33.0	0.042
Serum Triglyceride (mg/dl)	152.7±48.7	172.1±33.7	192.8±73.1	0.013
CRP (mg/L)	7.9±2.8	8.5±2.3	9.2±2.6	0.046
IL-6 (pg/ml)	24.3±11.1	28.8±12.2	34.1±10.7	0.001

P value reached from ANNOVA

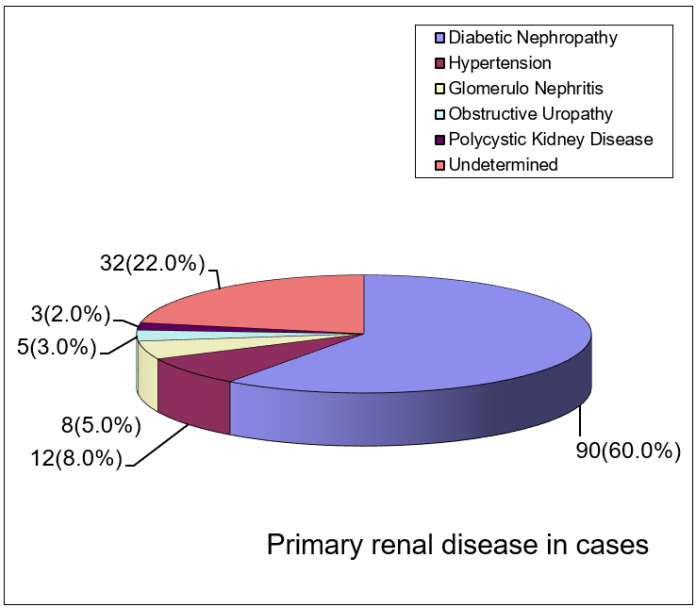

Figure 1: Pie chart shows primary renal disease of the cases

Table III is a frequency distribution table with mutually exclusive type class interval. CRP values were grouped into 5 classes. Number of the cases according to their cut off laboratory values and CRP level were mentioned in each class. Number of cases with Hb% (below ≤11 gm/dl) (n=54); serum total cholesterol (above ≥150 mg/dl) (n=116); serum triglyceride (above ≥150 mg/dl) (n=105) levels are not statistically significant (p>0.05) with CRP level. But number of cases with serum Ca^{++} (below ≤8.4 mg/dl) (n=106) and serum PO^4 (above ≥ 4.3 mg/dl) (n=113) levels are statistically significant (p<0.05) with CRP level. That indicates CRP is related with some of the factors responsible for CKD progression.

Table III: Distribution of the cases according to their cut off laboratory values and CRP level

	CRP (mg/L)										P value
	6.0-6.9 (n=21)		7.0-7.9 (n=39)		8.0-8.9 (n=42)		9.0-9.9 (n=22)		>10 (n=26)		
	n	%	n	%	n	%	n	%	n	%	
Hb% ≤11 (gm/dl) (n=54)	6	11.1	15	27.8	15	27.8	10	18.5	8	14.8	0.778
S. Ca^{++} ≤8.4 (mg/dl) (n=106)	15	14.2	24	22.6	30	28.3	22	20.8	15	14.2	0.012
S PO^4 ≥ 4.3 (mg/dl) (n=113)	19	16.8	31	27.4	31	27.4	11	9.7	21	18.6	0.026
Total cho. (≥150 mg/dl) (n=116)	17	14.7	27	23.3	33	28.4	18	15.5	21	18.1	0.720
Serum Tg (≥150 mg/dl) (n=105)	17	16.2	26	24.8	32	30.5	13	12.4	17	16.2	0.450

P value reached from chi square test

Table IV is a frequency distribution table with mutually exclusive type class interval. IL-6 values were grouped into 5 classes. Number of the cases according to their cut off laboratory values and IL-6 level were mentioned in each class. Number of cases with Hb% (below ≤11 gm/dl) (n=54); serum Ca^{++} (below ≤8.4 mg/dl) (n=106); serum PO^4 (above ≥ 4.3 mg/dl) (n=113) serum total cholesterol (above ≥150 mg/dl) (n=116) and serum triglyceride (above ≥150 mg/dl) (n=105); levels are statistically significant (p<0.05) with IL-6 level. That indicates IL-6 is related with the factors responsible for progression of CKD.

Table IV: Distribution of the cases according to their cut off laboratory values and IL-6 level

	IL-6 (pg/ml)										P value
	19.0-21.9 (n=18)		22.0-24.9 (n=39)		25.0-27.9 (n=40)		28.0-30.9 (n=30)		>31 (n=23)		
	n	%	n	%	n	%	n	%	n	%	
Hb% ≤11 (gm/dl) (n=54)	6	11.1	14	25.9	14	25.9	6	11.1	14	25.9	0.034
S. Ca^{++} ≤8.4 (mg/dl) (n=106)	15	14.2	25	23.6	22	20.8	21	19.8	23	21.7	0.002
S. PO^4 ≥ 4.3 (mg/dl) (n=113)	15	13.3	32	28.3	29	25.7	16	14.2	21	18.6	0.001
Total cho. (≥150 mg/dl) (n=116)	14	12.1	28	24.1	33	28.4	18	15.5	23	19.8	0.043
S. Tg (≥150 mg/dl) (n=105)	13	12.4	21	20.0	30	28.6	18	17.1	23	21.9	0.002

P value reached from chi square test

Table V shows comparison of mean eGFR, mean value of CRP and IL-6 level of the cases among different stages of CKD. It was observed that, eGFR is gradually declining but CRP and IL-6 were gradually increasing with the stages of CKD which is statistically significant ($p<0.05$). Between CRP and IL-6; IL-6 has more significant relation with stage progression of CKD.

Table V: Comparison of mean eGFR, mean value of CRP and IL-6 level of the cases among different stages of CKD

	CKD stage			P value
	Stage 3	Stage 4	Stage 5	
	Mean±SD	Mean±SD	Mean±SD	
eGFR (ml/min/1.73 m^2)	40.21±5.92	22.54±4.3	12.02±.98	0.001
CRP (mg/L)	7.9±2.8	8.5±2.3	9.2±2.6	0.046
IL-6 (pg/ml)	24.3±11.1	28.8±12.2	34.1±10.7	0.001

P value reached from ANNOVA

Table VI shows association of CRP and IL-6 level with eGFR. Correlation coefficient (r value) of eGFR vs. CRP is -0.592 that indicates there is weak negative correlation between CRP and eGFR and it is statistically significant ($p<0.05$). Correlation coefficient (r value) of eGFR vs. IL-6 is -0.613 which indicates the degree of negative association between IL-6 and eGFR is strong which is statistically very much significant ($p<0.05$).

Table VI: Association of CRP and IL-6 level with eGFR

	Correlation coefficient r value	P value
eGFR vs. CRP	-0.592	0.001
eGFR vs. IL-6	-0.615	0.001

Table VII: Multiple Regression analysis

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.606[a]	0.367	0.336	10.229

a. Predictors: (Constant), CRP, Hb%, Age (in years), S.Total Cholesterol, Serum Calcium, $S.PO_4$, S.Triglyceride

The regression model with independent variables CRP, Hb%, Age (in years), S.Total Cholesterol, Serum Calcium, S.PO4 and S.Triglyceride explain 36.7% of the observed variability in eGFR.

Coefficients[a]							
Model	Unstandardized Coefficients		Standardized Coefficiens	t value	p value.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	72.569	20.887		3.474	0.001	31.278	113.859
Age (in years)	-0.019	0.073	-0.018	-0.265	0.791	-0.164	0.125
Serum Calcium	-1.116	1.616	-0.048	-0.691	0.491	-4.311	2.078
S.PO4	0.223	1.503	0.010	0.148	0.882	-2.749	3.194
S.Total Cholesterol	-0.012	0.021	-0.041	-0.566	0.572	-0.054	0.030
S.Triglyceride	0.022	0.014	0.113	1.545	0.125	-0.006	0.051
Hb%	0.447	0.608	0.049	0.735	0.464	-0.755	1.648
CRP	-1.251	0.629	-0.575	-8.348	0.001	-6.494	-4.007
a. Dependent Variable: eGFR							

The intercept (constant) 72.569 is the value of eGFR when all independent variables are 0. In this cases CRP slope is -1.251, which indicates that CRP has a significant ($p<0.001$) negative association with eGFR after taking into account other factors (Age in years, Hb%, S. Total Cholesterol, Serum Calcium, $S.PO_4$, and S. Triglyceride) which can alter the eGFR val.

Table VIII: Multiple Regression analysis

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.843[a]	0.711	0.697	6.906

a. Predictors: (Constant), IL-6, Hb%, Age (in years), S.Total Cholesterol, Serum Calcium, S. PO_4, S.Triglyceride.

The regression model with independent variables IL-6, Hb%, Age (in years), S.Total Cholesterol, Serum Calcium, S.PO4 and S.Triglyceride explain 71.1% of the observed variability in eGFR.

Coefficients[a]							
Model	**Unstandardized Coefficients**		**Standardized Coefficiens**	**t value**	**p value**	**95.0% Confidence Interval for B**	
	B	**Std. Error**	**Beta**			**Lower Bound**	**Upper Bound**
(Constant)	94.681	13.979	0.0	6.773	0.001	67.047	122.315
Age (in years)	-0.038	0.049	-0.035	-0.77	0.443	-0.136	0.06
Serum Calcium	-0.845	1.086	-0.036	-0.778	0.438	-2.991	1.301
S.PO4	0.409	1.012	0.019	0.405	0.686	-1.59	2.409
S.Total Cholesterol	0.007	0.014	0.025	0.503	0.615	-0.021	0.036
S.Triglyceride	0.003	0.01	0.016	0.324	0.747	-0.016	0.023
Hb%	1.023	0.412	0.113	2.485	0.014	0.209	1.836
IL-6	-2.826	0.157	-0.842	17.956	0.001	-3.137	-2.515
a. Dependent Variable: eGFR							

The intercept (constant) 94.681 is the value of eGFR when all independent variables are 0. Here the slope is positive for Hb%, (1.023) which indicates that Hb increases and eGFR also increased. The other slope is negative and large for IL-6 (-2.826) which indicate that IL-6 increases but the other variable eGFR decreases and a small change in IL-6 would lead to a larger change in eGFR. Therefore IL-6 has a significant ($p<0.001$) negative association with eGFR after taking into account other factors (Age in years, Hb%, S.Total Cholesterol, Serum Calcium, $S.PO_4$, and S.Triglyceride) which can alter the eGFR value. In the comparison of two regression models the slope for IL-6 (-2.826) is more negative than CRP (-1.251) that indicates in the prediction of eGFR IL-6 has stronger negative association than CRP after adjusting the other factors, responsible for changes in the eGFR value.

DISCUSSION

One study stated that in the last decades of the 20th century, an exponential increase in the prevalence of ESRD, with its array of negative consequences at both the individual and population level has led, first to the identification of factors associated with a faster progression to CKD and second to interventions in those factors amenable to modification [1]. Interestingly interventions in some of these classical factors have proved to successfully delay the progression of CKD. Nevertheless and despite optimal intervention, patients still progress to ESRD, suggesting that pathophysiological mechanisms are involved in the natural evaluation of CKD. The search for markers of these other mechanisms has resulted in the findings of new biochemical markers attractive to further therapeutic intervention. With the similar views and objectives we have conducted this cross sectional study to measure serum level of CRP and IL-6 of patients in CKD stage 3-5 and to measure additional laboratory parameters for anaemia, dyslipidaemia and calcium haemostasis in CKD patients and also to see the association of those inflammatory markers with CKD stage denoted by eGFR and their relation to above mentioned laboratory parameters.

A total of 182 patients from outpatient department of Nephrology of National Institute of Kidney Diseases and Urology (NIKDU), Dhaka during January 2016 to December 2016, were included in this study. Among them, 150 predominantly patients of CKD with stage 3 to 5 and rest of 32 patients without CKD were enrolled in this study as cases and control respectively. Patients aged 18 years and above and diagnosed patients of CKD stage 3 to 5 were enrolled in this study. Patients suffering from acute kidney injury, patients had history of dialysis, kidney transplant, patients having acute infection, malignancy or liver disease, patients receive immunotherapy or chemotherapy in recent period (<6 months), and patients having history of connective tissue disease were excluded from the study. Age and sex matched group with eGFR≥60 ml/min/1.73m² with normal urine routine and microscopic examination (absence of RBC, protein and casts) were taken as control. The present study findings were discussed and compared with previously published relevant studies.

The present study shows that inflammatory biomarkers CRP and IL-6 both are significantly elevated in patients in patients with CKD compared to controls without CKD. But the rise of IL-6 is more rapid and more significantly associated with the severity of CKD measured by eGFR than CRP. Lee *et al.*, (2015) in their study showed that inflammatory biomarkers like TNF-α, IL-6 and CRP were significantly elevated in patients with CKD. After taking into consideration other risk factors for CKD, comorbid conditions and use of medications like antihypertensive, antidiabetics, lipid lowering agents, and aspirin CRP level was not significantly elevated in predialysis CKD patients. That is serum level of CRP is not independently associated with CKD. But in patients with CKD compared to controls the level of IL-6 and TNF-α were independently associated with the

severity of CKD. This finding is consistent with our study.

In our study 60% of our CKD patients had diabetic nephropathy as a primary cause. In 22% cases of CKD primary cause is undetermined. 8% of our CKD cases had hypertension and only 5% had glomerulonephritis. The prevalence of CKD Stages 2–5 has continued to increase since 1988 as have the prevalences of diabetes and hypertension, which are respectively etiologic in approximately 40% and 25% of CKD cases. 7 Other study found 17.0% DM and 14.0% hypertension in their study. 10 Our findings regarding the aetiology of CKD, diabetes mellitus is the leading cause which is consistent with other studies. But the prevalence of both undetermined factors and glomerulonephritis as aetiology of CKD are not consistent with other studies. This finding is due to the lack of availability of renal biopsy reports. Cases who are suspected to have glomerulonephritis from clinical, ultrasonography and urine microscopy findings lack the renal biopsy reports. They are being categorized in undetermined aetiology group rather than glomerulonephritis group.

Regarding age (53.2±11.3 yrs) and sex (male predominance) distribution of the cases, our study is similar with Zhang *et al.*, (2012). Similarly other studies also found male predominance in their studies. 9-10 Another study reported that mean BMI significantly higher in CKD patients compared to controls which is comparable with the current study.11 Majority of our study population belongs to stage 3 (37%) of CKD, which is similar with one study having 44.6% of the patients in stage 3. 1 In our study there is a statistically significant difference in the mean value of eGFR and different laboratory parameters (s.creatinine, Hb%, S.Ca^{++}, S.PO_4, S.total cholesterol, S.triglyceride) at stage 3-5 of CKD that is they are related with the stage progression of CKD. Anemia of CKD and CKD-Mineral Bone disorder (CKD-MBD) often begin during stage 3. Hypertension is aggravated in CKD stages 3-5 and acid base balance, dyslipidaemia and glucose homeostasis become degraded later. This statement is compatible with the findings of our study.

Among the factors responsible for CKD progression we have taken into account hemoglobin content, serum Ca^{++}, serum PO_4, serum total cholesterol and serum triglyceride level. These laboratory parameters are selected as they are modifiable. One of the objectives of our study is how CRP and IL-6 is related with these parameters. Our study shows that the number of patients who have hypocalcaemia and hyperphosphataemia and their relation with CRP level is statistically significant but the relation of CRP level with the number of patients having anemia, hypercholesterolemia and hypertriglyceridemia is not significant statistically. That is CRP is related to some of the factors responsible for CKD progression. After taking into account the IL-6 level, in our study we find the relation of IL-6 level with the numbers of CKD patients having anemia, hypocalcaemia, hyperphosphataemia, hypercholesterolemia and hypertriglyceridemia and that relation is statistically significant. That indicates IL-6 level is statistically related with the factors responsible for CKD progression.

Other study in their study showed that anemia and IL-6 an important marker of inflammation, independently predicted faster progression to ESRD in pre dialysis CKD patients which is consistent with our findings [12]. Lipid disorders that predict death are decreased HDL, increased triglycerides, or an increased triglyceride/HDL ratio. Total cholesterol is actually inversely related to mortality, suggesting that qualitative changes in lipid composition or in the endothelium are more important than total lipid levels, especially the classic paradigm of increased LDL cholesterol leading to vascular disease [2]. Another study reported that serum Cholesterol, Triglyceride and LDL-C among all groups were significantly increased when compared with control $p<0.05$. Serum HDL-C among all study groups was significantly decreased $p<0.05$, along with increase in LDL/HDL ratio among all study groups when compared with control $p<0.05$ [11]. The investigators concluded that dyslipidemia occurs gradually in CKD patients as disease progresses.

In our study we included only the predialysis CKD patients and we did not see the association of LVH with CRP and IL-6. Degree of proteinuria has previously been shown to be an important predictor of progression of renal disease. But in the study 24-hour urine protein level did not emerge as an independent predictor of ESRD [2]. In our study we do not have the available 24hour urine protein reports and we could not see the association of CRP and IL-6 level with 24hour urine protein level. Collection of urine for 24 hours is little bit difficult. Though it is needed some patient did not agree to perform the report make the test unavailable in our study.

Our study shows that CRP level significantly elevated with the decline in eGFR. CRP is a well-recognized risk factor for CVD and mortality in the general population as well as in patients with ESRD [3-5]. However, in other study indicates that circulating CRP level was not significantly elevated in pre-dialysis CKD patients after accounting for established CKD risk factors, history of CVD, and use of antihypertensive, antidiabetic, and lipid-lowering agents, and aspirin [2].

Observation of this present study is that, the level of IL-6 is significantly higher with the decline in eGFR. Another study reported that plasma levels of IL-1b, IL-1RA, IL-6, TNF-α, hs-CRP, and fibrinogen were higher among participants with lower levels of eGFR. Inflammation score was higher among those with lower eGFR and higher UACR. Each unit increase in eGFR,

cystatin C, and UACR was associated with a 21.2% (95% confidence interval, 21.4, 21), 64.9% (56.8, 73.3) and 0.6% (0.4, 0.8) change in IL-6, respectively (P<0.05) [11].

Another study reported that the IL-6 levels tended to rise as CKD progressed with the increase becoming statistically significant at CKD stages 5 and 5D. Further examination confirmed an inverse linear relationship between IL-6 levels and the eGFR when the analysis was restricted to pre-dialysis CKD patients at stages 2–5 which is consistent with our study [3]. Now why the level of IL-6 is raised with the decline in eGFR and what is its clinical consequences? Pecotis- Filho *et al.*, (2003) in their study stated that the potential causes of elevated plasma IL-6 levels in ESRD patients may be related to the loss of kidney function, uraemia per se (and its sequelae, such as fluid overload, oxidative stress and susceptibility to infections) and dialysis-related factors. Even before the initiation of dialysis therapy, patients with decreased renal function already demonstrate signs of inflammation and the deterioration of renal function has been associated with a significant increase in serum cytokine levels.

In our study we also use the multiple regression model to predict the eGFR value from the level of CRP and IL-6. Multiple regression analysis shows regression coefficient (B) for CRP and IL-6 is 1.39 and -3.58 respectively that indicates in the prediction of eGFR IL-6 has a negative larger slope than CRP. That means CRP had small effect on eGFR. Therfore IL-6 (p=.001) had significantly more association with eGFR than CRP(p=0.036). in another study in a case control study found that median serum levels of CRP, IL-6 and TNF-α were significantly higher in patients with CKD compared to controls. After adjusting for important co-variable the median of IL-6 and TNF-α but not CRP remained significantly higher. Finally the findings of our study coincides with this study [6].

Potential limitations of this study should be noted. First, this study cannot establish a temporal relationship or make causality inferences due to its cross-sectional, observational nature. Second, the study included a single measurement of the proinflammatory cytokines, which may not represent average levels of these biomarkers over time. However, patients with acute kidney injury were excluded from the present study. In addition, serum creatinine-based eGFR was used to define kidney function in our study. The use of cystatin C-based or cystatin- and creatinine based eGFR might provide a better assessment of kidney function regarding its predictive value for the risks of death and ESRD. Finally, we were not able to analyze the association between inflammatory markers and subtype of CKD by different causes because renal biopsy data were not available in our study.

CONCLUSION

In conclusion inflammatory marker CRP and IL-6 both are significantly raised in advanced CKD stage 3-5. IL-6 is more rapidly increasing and more strongly associated with advancing stages 3-5 of CKD than CRP.

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