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RESEARCH ARTICLE

Prognostic Potential of Serum Biomarkers as Predictors for Cardiovascular Complications and Disease Progression in Chronic Kidney Disease Patients

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ABSTRACT:
Aim: Chronic Kidney Disease (CKD) is characterized by progressive loss of kidney function over a period of time. The aim of the study is to determine the prognostic potential of serum biomarkers for cardiovascular complications and disease progression in chronic kidney disease patients.
Materials and Methods: This retrospective observational study was carried out in the nephrology department of a multispecialty hospital for a period of two months. Clinical and biochemistry reports of 499 CKD patients were collected in designed case report forms. All statistical analysis was carried out using International Business Machine (IBM) Statistical Package for Social Sciences (SPSS) 17.0.
Results: A direct correlation was observed between cardiac enzyme markers and phosphate levels. The product of Glycated Hemoglobin (HbA1c) and number of years of Diabetes Mellitus (DM) as predictive risk factors for development and progression of nephropathy (DN-CKD).
Conclusion: This study has attempted to evaluate altered serum biomarkers as potential prognostic factors of cardiovascular complication and progressive renal impairment in CKD patients. Further prospective studies are required to gain deep insights into this and thereby aid in making significant clinical decisions.

KEYWORDS: CKD, cardiomyocyte injury, biomarkers, hyperphosphatemia, diabetes

INTRODUCTION:
Chronic Kidney Disease is characterized by progressive loss of kidney function over a period of time. Progressive loss of kidney function leads to uremic waste accumulation which is marked by elevated endogenous renal parameters [1]. Diabetes mellitus is one of the major causes of renal failure. Approximately 30% of patients with diabetic nephropathy eventually progress to end-stage renal failure and the rest usually die from cardiovascular disease before reaching end stage [2]. Azotemia in turn may provoke diverse complications in other systems, the prime significant being cardiovascular system [3]. In addition, altered hemodynamics and serum biomarkers mark CKD stages and could therefore predict cardiovascular complications and disease progression [4].

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Absolute eosinophil count (AEC) is the product of percentage eosinophils and white blood cell count and serves as a potential indicator of cardiovascular complication. AEC increases with CKD progression: higher AEC values being observed in stage IV and End Stage Renal Disease (ESRD) patients. AEC values are also increased in CKD patients with cardiovascular comorbidity [5, 6].

C-reactive protein (CRP) is a peptide that serves as an acute phase inflammatory marker. Higher CRP values are known to be associated with cardiovascular complications. However, being a macromolecular peptide, filtration of CRP is decreased in progressive loss of kidney function. Thus, increased CRP values are supposed to be observed in higher CKD stages and could therefore predict CKD progression [7, 8]. Hence, we evaluated the correlation of CRP levels with Glomerular Filtration Rate (GFR) which serves as an endogenous marker of kidney function. D-dimer is a breakdown

peptide formed from fibrinolysis. Elevated D-dimer, prothrombin time values are observed in CKD patients due to platelet dysfunction and thrombocytopenia [9]. Thus, evaluation of correlation of D-dimer levels with GFR could serve as a predictive marker for CKD progression. Further, platelet dysfunction may contribute to altered hemostasis and therefore measuring D-dimer values could also be used for prognosis of vascular and bleeding complications in CKD patients [10].

Diabetic nephropathy (DN) is major cause of CKD in patients presented for renal replacement therapy. Increased systemic glucose load contributes to renal tubular damage due to increase in the amount of glucose presented to the kidneys for filtration [11, 12]. However, acute onset of renal damage due to diabetes mellitus is not evident and occurs with increase in number of years on DM. Thus, DM induced CKD stages may correlate to some extent with blood sugar profile and duration of renal tubules exposed to increased systemic glucose load. Decreased renal function contributes to increase in electrolyte retention.

Hypertension is a common complication CKD. Besides sodium and potassium, various other cationic electrolytes are retained in systemic circulation, phosphorus being one such electrolyte. Hyperphosphatemia induces cardiac myocyte damage [13, 14] and hence cardiac enzyme markers have been shown to be increased in stage IV and stage V CKD patients. Thus, evaluation of correlation between cardiac enzymes (Creatine Kinase MB [CK-MB], Creatine Phosphokinase [CPK], Troponin T) and phosphorus in stage IV and ESRD patients could provide potential prognostic markers for acute myocardial injury in CKD patients. Hence the study was designed to determine the prognostic potential of serum biomarkers for cardiovascular complications and disease progression in chronic kidney disease patients.

MATERIALS AND METHODS:

This retrospective observational study was carried out in the nephrology department of a multispecialty hospital for a period of 2 months from January 2015-March 2015.The study protocol was approved by the institutional ethics committee of Vels University (Approval no: IEC/DOP/2015/05). Consent from the hospital authorities and nephrologists were obtained before accessing the clinical records of patients. Clinical data was recorded from the patient case sheets stored in medical records department of the hospital whereas biochemical parameters were recorded from the laboratory database. All the clinical and biochemistry data were recorded in a predesigned case report form. 499 CKD patients who fulfilled the inclusion and exclusion criterion were recruited retrospectively for participation in the study. Patients of both gender who visited the hospital for hemodialysis or routine investigation as instructed by the consulting nephrologist were included whereas renal complications other than CKD such as acute kidney injury, renal calculi and case sheets with insufficient clinical data were excluded.

Statistical Analysis:

Determination of predictors of disease progression in CKD patients with DM was done using logistic linear regression models. Pearson's correlation was used to determine the linear dependency of variables. All statistical analyses were performed using IBM SPSS 17 statistics package.

RESULTS:

The study population for the retrospective analysis included chronic kidney disease patients of both genders who visited the hospital for any of the following reason: Hemodialysis or routine medical checkup as instructed by the consulting nephrologist or any other comorbidity. Age wise distribution of patients considered for the study is shown in Table 1. 64% patients were males whereas 36% patients were females.

GFR is an endogenous marker of renal function that requires 24 hours urine collection. However, GFR can theoretically be estimated using the modification of diet in renal disease (MDRD) formula from serum creatinine and age of the patient. CKD patients in the study have been segregated into different GFR quartiles as shown in Table 2.

Table 1: Age Wise Segregation of CKD patients

Age Interval	No. of Patients (N=499)	Percentage (%)	Mean	SD	Age Quartiles	Median Age
11-20	13	2.61	17	2.07	13-20	17
21-30	24	4.81	26	2.38	21-30	25.5
31-40	49	9.82	35.97	3.15	31-40	37
41-50	69	13.83	46.44	2.52	41-50	47
51-60	146	29.26	55.58	2.84	51-60	55.5
61-70	116	23.25	65.25	2.56	61-70	65
71-80	59	11.82	75.45	2.35	71-80	75
81-90	23	4.61	83.68	2.80	81-90	82.5

Mean age = 55.87±15.59, Median age = 57

Table 2: Segregation of Patients based on Estimated GFR

GFR(ml/min/1.73m$^{2)}$	NO. OF PATIENTS (n=499)	% OF PATIENTS
STAGE 1	9	2
STAGE 2	27	5
STAGE 3	80	16
STAGE 4	105	21
STAGE 5	278	56
TOTAL NO. OF PATIENTS	**499**	**100**

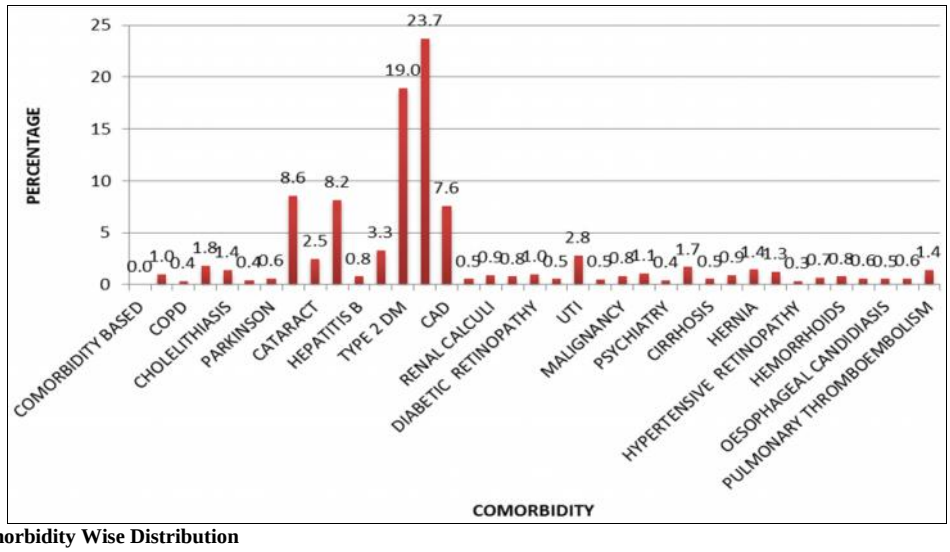

Figure 1: Co morbidity Wise Distribution

The co morbidities observed in renal transplant recipients taken for the study are shown in Figure 1.

Estimated GFR was correlated with age to determine the linear dependency using Pearson's correlation. The results are shown in Figure 2.

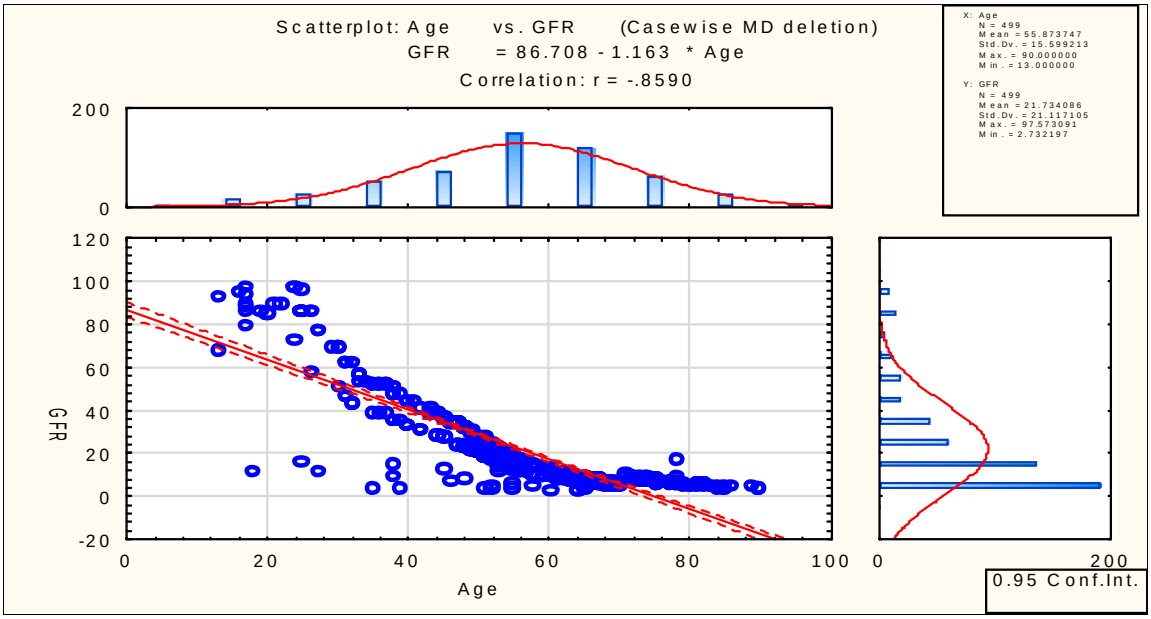

Figure 2: Correlation of Estimated GFR with Age (Pearson's Coefficient)
y = -1.1629x + 86.708 R^2 = - 0.8590, correlation significant at 0.01 level (two-tailed).

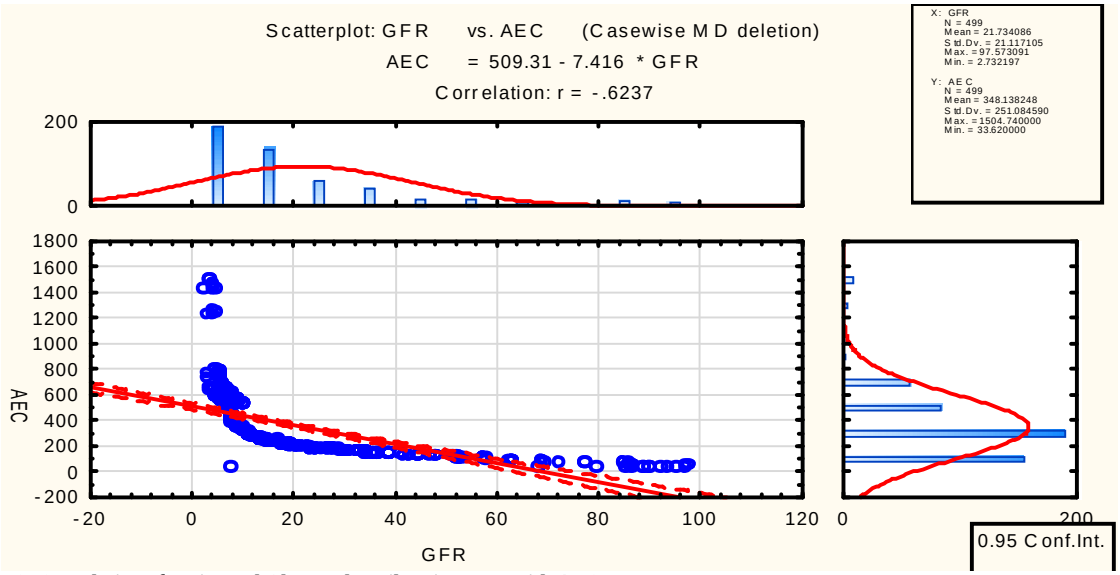

Figure 3: Correlation of Estimated Glomerular Filtration Rate with AEC
y = -7.4155x + 509.31 R^2 = -0.624 Correlation is significant at the 0.01 level (2-tailed).

Significant inverse correlation was found between GFR and age with a correlation coefficient of -0.8590 Estimated GFR was correlated with AEC to determine the linear dependency using Pearson's correlation. The results are shown in Figure 3.

Significant inverse correlation was found between GFR and AEC with a correlation coefficient of -0.624 Estimated GFR was correlated with AEC in CKD patients with cardiovascular complications to determine the linear dependency using Pearson's correlation. The results are shown in Figure 4.

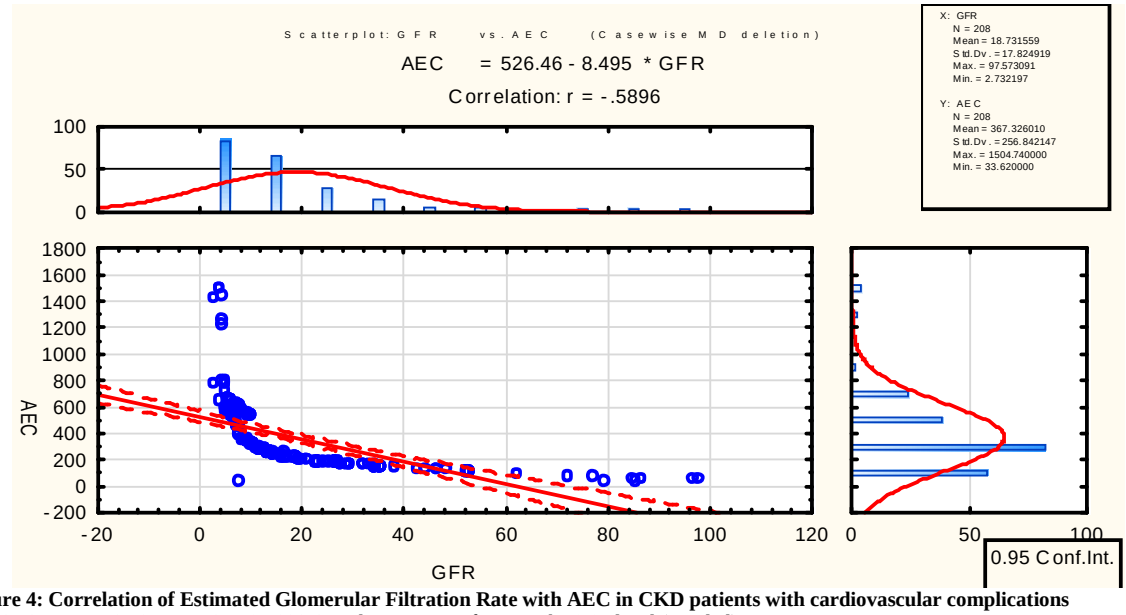

Figure 4: Correlation of Estimated Glomerular Filtration Rate with AEC in CKD patients with cardiovascular complications
y = -8.4954x + 526.46 R^2 = -0.5896 Correlation is significant at the 0.01 level (2-tailed).

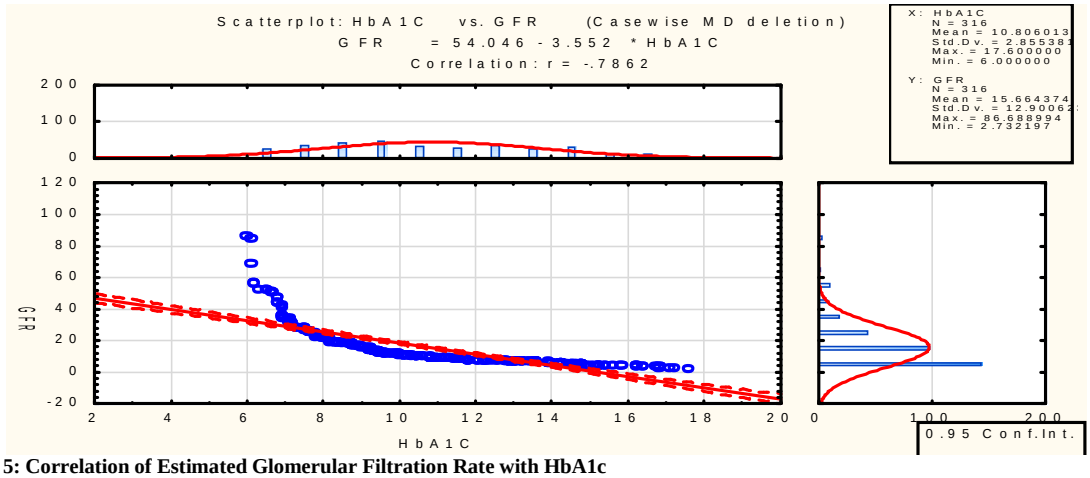

Figure 5: Correlation of Estimated Glomerular Filtration Rate with HbA1c
y = -0.174x + 13.532 R^2 =-0.786 Correlation is significant at the 0.01 level (2-tailed).

Significant inverse correlation was found between GFR and AEC with a correlation coefficient of -0.5896 Estimated GFR was correlated with HbA1c to determine the linear dependency using Pearson's correlation. The results are shown in Figure 5.

Significant inverse correlation was found between GFR and HbA1c with a correlation coefficient of -0.786

Logistic linear regression models were used to determine the predictor of DN-CKD. The results are given in Table 2 and the coefficients are summarized in Table 3.

Estimated GFR was correlated with CRP to determine the linear dependency using Pearson's correlation. The results are shown in Figure 6.

Table 2: MLR_ Predictors of CKD Progression in DM Patients

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.944a	.890	.890	4.2827946

a. Predictors: (Constant), Years on DM, HbA1C

Table 3: Coefficient of Predictors of CKD Progression in DM Patients

Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	66.041	1.038		63.643	.000
	Years on DM	-1.538	.055	-.667	-27.905	.000
	HbA1C	-1.673	.108	-.370	-15.483	.000

Dependent Variable: GFR

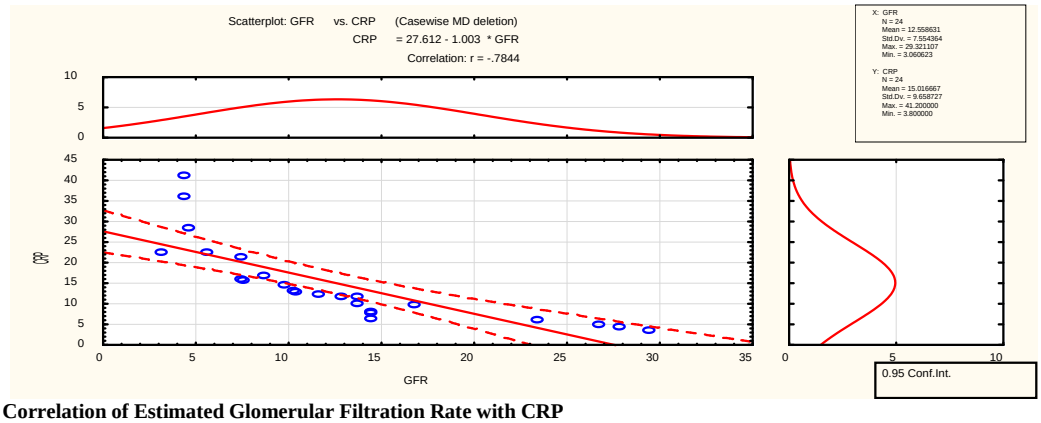

Figure 6: Correlation of Estimated Glomerular Filtration Rate with CRP
y = -1.0029x + 27.612 R^2 = -0.7844 Correlation is significant at the 0.01 level (2-tailed).

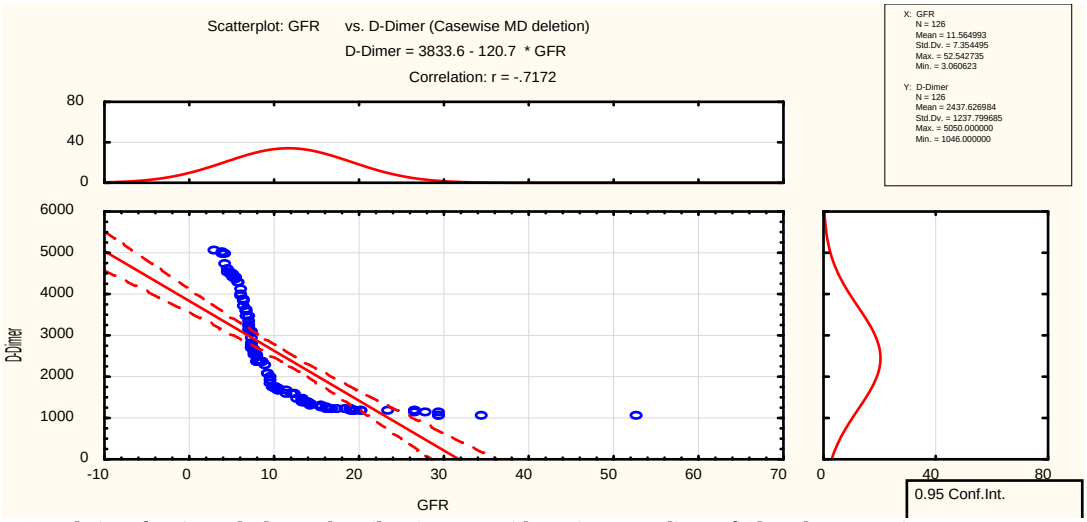

Figure 7: Correlation of Estimated Glomerular Filtration Rate with D-Dimer- Predictor of Altered Hemostasis
y = -120.7x + 3833.6 R^2 = -0.7172 Correlation is significant at the 0.01 level (2-tailed).

Significant inverse correlation was found between GFR and CRP with a correlation coefficient of -0.7844 Estimated GFR was correlated with D-Dimer to determine the linear dependency using Pearson's correlation. The results are shown in Figure 7.

Significant inverse correlation was found between GFR and D-dimer with a correlation coefficient of -0.7172 Serum phosphorous was correlated with cardiac enzymes to determine the linear dependency using Pearson's correlation. The results are shown in Table 4 and Figure 8, 9, 10.

Table 4: correlation of serum phosphorous with cardiac enzymes

Parameter	Correlation Coefficient
CK-MB	0.850*
CPK-Total	0.835*
Troponin T	0.581*

CK-MB was found to be highly elevated when compared with other cardiac enzymes with a correlation coefficient of 0.85

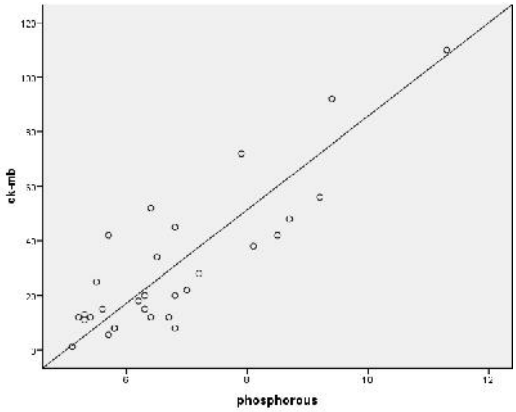

Figure 8: Correlation of Phosphorous with CK-MB

Significant positive correlation was found between phosphorous and CK-MB with a correlation coefficient of 0.850

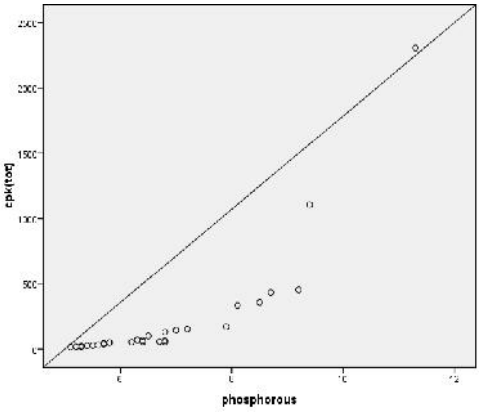

Figure 9: Correlation of Phosphorous with CPK-Total

Significant positive correlation was found between phosphorous and CPK-Total with a correlation coefficient of 0.835.

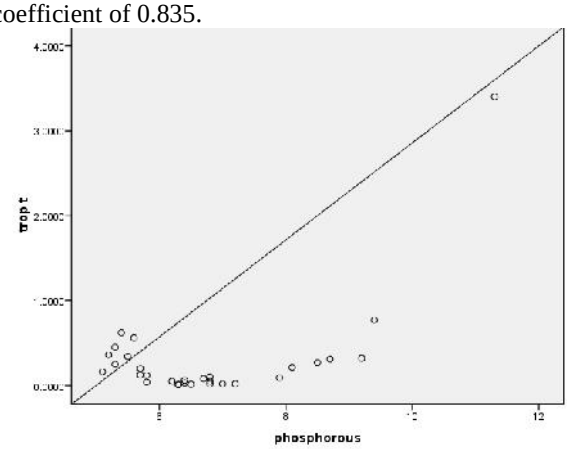

Figure 10: Correlation of Phosphorous with Troponin-T

Significant positive correlation was found between phosphorous and CK-MB with a correlation coefficient of 0.581

DISCUSSION:

Renal function impairs as age progresses [15]. This is marked by an increase in renal endogenous markers such as Serum creatinine and Uremic accumulation. Thus a linear relationship exists between age and Serum creatinine (Perfect positive correlation). However, under renal disease conditions, the levels of endogenous markers follow non-linear kinetics and hence a near positive or no correlation may only be observed when attempted to linearly correlate age with endogenous marker levels. The near positive correlation observed is attributed to hemodialysis and younger patients with CKD stages above III. Diabetes mellitus is a condition characterized by hyperglycemia and glycosuria. Increased systemic glucose load leads to increase in amount of glucose presented to the renal tubules to be filtered. Thus, progressive renal damage occurs with uncontrolled diabetes mellitus[16].

However, linear dependency of GFR cannot be observed when correlated with systemic load of glucose as the univariate. Hence, the product of number of years of diabetes and glycosylated hemoglobin was correlated with estimated glomerular filtration rate. A near negative correlation (near-inverse) was observed, suggesting that in diabetic patients, uncontrolled hyperglycemia and number of years as predictive risk factors for development and progression of nephropathy (DN-CKD). Cardiovascular complication such as cholesterol embolization are often under diagnosed in chronic kidney disease patients since they only clinically manifest as non-specific inflammatory responses such as hypereosnophilia and elevated ESRD [5]. It has been reported that elevated AEC values are observed in patients with higher CKD stages. Hence we statistically correlated calculated AEC values with GFR in CKD patients. In contrast to the expected result, AEC was found to be significantly elevated in stage IV and ESRD patients with cardiovascular complications and decreasing renal function. Thus, AEC could be used to prognoses developing cardiovascular complications in CKD patients and also monitor disease progression. Thrombocytopenia, glomerular thrombosis and thrombi in small arteries and glomerular capillaries are common pathologic features of CKD. Platelet dysfunction in CKD and ESRD is also due to both intrinsic platelet abnormalities and impaired platelet vessel-wall interaction [9]. Nearly 60% of patients with a central venous catheter for dialysis develop thrombosis. Disturbance of coagulation and fibrinolysis has been reported in patients with chronic kidney disease [17]. It is reasonable to assume that the higher levels of D dimer are primarily as a result of increased fibrin clot formation and breakdown.

The increased thrombogenic state may be related to increased susceptibility to vascular disease in these patients. Correlation of observed d-dimer values of with GFR in CKD patients with atherosclerosis of coronary arteries showed an inverse correlation. Hence D-dimer is a potential marker for predict vascular complications in CKD patients. CRP is an acute phase inflammatory marker whose values are known to be elevated in patients with atherogenic diseases. However, being a macromolecular peptide its clearance is reduced in CKD patients. We have observed near inverse correlation between CRP and GFR in a very limited sample of CKD patients with co-morbid CAD [7]. However, these CRP values may duly be influenced by comorbid CAD, and hence further prospective studies are required to evaluate the effectiveness of CRP in predicting CKD progression. A direct correlation was observed between cardiac enzyme markers and phosphate levels, suggestive of increased phosphate retention with progressive renal impairment.

CONCLUSION:

Chronic kidney disease is often associated with various cardiovascular complications besides renal insufficiency. Various hemodynamic and peptide markers are altered in chronic kidney disease due to impaired clearance and comorbid secondary complications. This study has attempted to evaluate altered serum biomarkers as potential prognostic factors of cardiovascular complication and progressive renal impairment in CKD patients. Further prospective studies are required to gain deep insights into this and thereby aid in making significant clinical decisions.

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