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Role of surrogate risk biomarkers for cardiovascular risk prediction in chronic kidney patients

Bhamidipaty Kanaka Durgaprasad

Professor, Department of Radiodiagnosis, GITAM Institute of Medical Sciences and Research, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Rama Rao Malla

Professor, Cancer Biology Lab, Dept of Biochemistry and Bioinformatics, GIS, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Corresponding author email: dr.rrmalla@gmail.com

Bhamidipaty Durgananda Lahari

III Year B.Tech Biotechnology, GIT, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Bhamidipaty Veenadhari

I Year B.Tech Computer Science, GIT, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Payala Vijayalakshmi

Assistant Professor, Department of Microbiology, GITAM Institute of Medical Sciences and Research, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Kolli Viswa Kalyan

Professor, Department of Biochemistry, GITAM Institute of Medical Sciences and Research, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

Abstract---Background: Biomarkers are quantifiable and repeatable biological indicators and some of them are used to predict cardiovascular events in individuals with chronic renal illness. Objectives: To assess, surrogate risk biomarkers like H-FABP, CIMT, serum albumin and A/C ratio in development of cardiovascular disease in stage III and stage IV CKD patients. Methods: It is a case control study, with sample size each of hundred cases and controls

who were recruited from Nephrology department and present with chronic kidney disease – stage III / IV. B-model ultrasonography was employed for CIMT evaluation. H-FABP would be estimated using ELISA. Serum albumin in blood samples was determined using the bromocresol green. Urinary Albumin/creatinine ratio was expressed as milligram of albumin excreted per gram of urinary creatinine. Results: It was found that, there was a mean increase in the values of H-FABP and CIMT for cases compared to control population. However there was no much increase in mean values for serum albumin to control and case population. Conclusion: use of established and significant laboratory biomarkers like H-FABP, CIMT, ACR, Serum albumin concentration were really proven to be assessing the CKD risk in the general population.

Keywords---CKD, cardiovascular disease, H-FABP, CIMT, ACR.

Introduction

Regardless of the underlying aetiology, chronic kidney disease is defined as an eGFR of less than 60mL/min per 1.73m² and/or a 3-month change in kidney damage marker levels. Cardiovascular disease is one of the primary causes of death and morbidity in hemodialysis patients, accounting for 50% of all deaths and morbidities. Both atherosclerosis and arteriosclerosis are found in people with end-stage renal disease (ESRD). To detect cardiovascular events in individuals with chronic renal illness, many biomarkers are used. Biomarkers are quantifiable and repeatable biological indicators^[1]. These biomarkers are objectively investigated and evaluated as indicators of normal biological processes, pathological processes, and pharmacologic responses to therapeutic interventions. The surrogate marker carotid intima-media thickness (CIMT) is largely used in dialysis patients to determine their atherosclerotic condition, because CIMT is strongly associated to cardiovascular disease (CVD) in chronic renal disease patients (CKD)^[2]. Although lipids are required for renal cell function, lipid metabolism disruption causes an inflammatory response, oxidative stress, fibrous degeneration, and necrosis in lean tissue. Fatty acid binding proteins (FABPS) are involved in lipid metabolism. Non-enzymatic cytosolic FABPS are abundant in animal tissues with a high rate of fatty acid metabolism. Heart-type (H)-FABP or FABP3 is found mostly in the heart, with less expression in other organs such as the kidney, skeletal muscle, testis, and stomach^[3]. FABPS is expressed in approximately 10 isoforms in various mammalian organs and cells. The majority of previous research has focused on H-importance FABP's in the heart, while H-involvement FABP's in the kidney has remained largely unknown. FABPs are a type of transport protein found in the cytoplasm that allows fatty acids to move through membranes^[3]. H-FABP levels were found to be associated with a greater number of cardiovascular risk factors and to be an independent risk factor for all-cause and cardiovascular death. Serum albumin, a negative acute phase reactant and marker for underlying inflammation and/or malnutrition, is an independent predictor of CVD and mortality in stage III and stage IV CKD patients. Low blood albumin levels are an unrecognized risk factor for kidney failure^[4]. Literature survey had showed that even in the small decline

levels of serum albumin will be strongly correlated with CVD, heart failure and mortality. The assessment of biomarkers to detect CVD events in renal injury has been studied before, but the sensitivity and specificity of H-FABP, CIMT, albumin levels, Albumin/creatinine ratio (A/C ratio) are still need to be improved. In the present study, the authors were intended to assess, some of the surrogate risk biomarkers like H-FABP, CIMT, serum albumin levels and A/C ratio in the development of cardiovascular disease in stage III and stage IV CKD patients.

Materials and Methods

Study design: It is a case control study.

Study population and Sample size

Includes sample size of one hundred patients of cases and one hundred patients of controls who recruited from the Nephrology department and present with chronic kidney disease – stage III / IV. The work was conducted in a Tertiary care teaching hospital, Visakhapatnam during the period of June 2019 to Feb 2021.

Patients

Inclusion criteria

- Estimated GFR (eGFR) < 60 ml/min/1.73 m² for ≥ 3 months.
- Age matched individuals without chronic kidney disease will be selected in control group.
- The proposed patients would be in the age group of 30 – 60 years and both males and females with CKD stage III & IV were included in the study.

Exclusion Criteria

- Patients with renal disease secondary to lupus nephritis or antineutrophil cytoplasmic autoantibody-associated vasculitis
- Patients with Nephrotic syndrome
- Patients with signs of acute infection, with recent history of liver failure, trauma, surgery, cancer or pregnancy
- Patients on glucocorticoids, immunosuppressant or anticoagulant medication in past 1 month.
- Patients with a history of previous thromboembolic or haemorrhagic events within the past 12 months.
- History of cardiovascular disease

Ethics approval

Institutional Ethics Committee clearance approval (No.GIMSR/Admn./Ethics/approval/IEC-10/2020) was obtained before the start of the study. Each participant was explained about the details of the study and informed consent obtained.

Methodology

The subjects' general conditions (age, gender, height, weight, systolic blood pressure, diastolic blood pressure and smoking history); underlying diseases (coronary heart disease (CHD) and diabetes mellitus) will be recorded. Fasting venous blood samples (5mL) will be collected under aseptic conditions from the study group after taking informed consent.

Measurement of Carotid intima-media thickness (IMT)

B-mode ultrasonography of the right and left near and far walls of the internal and common carotid arteries was employed for the CIMT evaluation. The maximum IMT of the internal and common carotid sites was calculated using the mean of the maximum IMT of the near and far walls of the right and left sides. The thickness of the carotid intima-media was measured using an ultrasound machine with Doppler capabilities (transducer probe frequency of 7.5 MHz). The intima-media thickness of both common carotid arteries on longitudinal views were measured at three distinct points about 1 cm proximal to the bulb with the individual in a supine position, neck extended, and head rotated 45° away from the side being scanned. The distance between the leading edge of the luminal echo and the leading edge of the adventitia of the media was defined as the intima-media thickness (IMT).

With a high-resolution B-mode ultrasound transducer, longitudinal pictures of the right and left common carotid arteries, carotid bulbs, and internal carotid arteries were collected and recorded for later analysis at the core laboratory. The distance between the luminal-intima interface and the medial-adventitial interface was measured using electronic callipers at several sites on the near and far walls of the distal 1 cm segments of the common carotid arteries, carotid bulbs, and internal carotid arteries. The mean IMT was calculated as the average of all IMT measurements; the mean of maximum IMT was calculated as the average of maximum wall thickness measurements from each region. The thickness of the carotid intima-media is a well-validated research instrument. If the thickness of CIMT was larger than 0.8 mm, it was deemed thickened in this study. In order to complete the procedures, the examiner was blinded to both the subjects and the controls.

Estimation of H-FABP

H-FABP would be estimated using ELISA. Solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. Samples and standards will be incubated together with peroxidase-conjugated second antibody in microtitre wells coated with antibodies recognizing human H-FABP. During incubation human H-FABP would be captured by the solid bound antibody. The secondary antibodies will bind to the captured human H-FABP. The peroxidase-conjugated second antibody will react with the substrate, tetramethylbenzidine (TMB). The enzyme reaction will be stopped by the addition of oxalic acid. The absorbance will be measured at 450 nm. A standard curve will be obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human H-FABP standards (log). The human H-FABP concentration of samples,

which are run concurrently with the standards, will be determined from the standard curve.

Estimation of Serum albumin by Immuno-turbidimetric assay

Serum albumin in blood samples was determined using the bromocresol green method. The serum albumin concentration were analyzed as a continuous variable per standard deviation (SD) and a categorical variable divided into quartiles (>4.21, 4.01-4.21, 3.81-4.00, ≤3.80).

Albumin: creatinine ratio (ACR)

It is ratio of urinary albumin to urinary creatinine; usually it is expressed as milligram of albumin excreted per gram of urinary creatinine. In our laboratory urinary albumin and creatinine concentrations are measured as (mg/dl). ACR is reported in (mg/g). Albumin concentration in spot urine sample is reported as UAC (mg/L) and in 24hour urine is reported as UAE (mg/24 hours). Urinary albumin excretion rate in timed urine is expressed as AER (µg/min). ACR (mg/g) can be calculated by albumin (mg/dl) divided by creatinine (g/dl). Calculations (formulae):

$$\text{ACR (mg/g)} = \text{Albumin (mg/dl)} / \text{Creatinine (mg/dl)} \times 1000.$$

Statistical analysis

Data was analysed statistically using Statistica 24.0 version. Results were presented in tabular form. Univariate analysis was used in description of demographic characteristics of the study population. Chi-square test will be used to know if all the parameters together are significantly better predictors of cardiovascular events in CKD patients. Chi-square test was used to determine the significant associations between categorical variable. Pearson's correlation was used to determine association between all the multiple variables P values < 0.05 were considered significant.

Results

This study involved 100 CKD stage III and IV patients and 100 control subjects without CKD. The CKD III and IV group comprised of 60 (60%) males and 40 (40%) females whereas control group contains 68 (68%) males and 32 (32%) females. The mean age group of CKD patients was 54.54 years and control group was 54.07 years (Table 1). Table 2 showed the Chi² paired t-test analysis results of study variables. It was found that, there was a mean increase in the values of H-FABP for cases (5.40) compared to control population (2.39). It was also observed that, there was a rise in the mean values of CIMT from control to case population (0.6 to 0.8). However there was no much increase in the mean values for serum albumin concentration to control and case population. There was a great variation in the mean values of ACR for control and test groups. By performing Pearson's correlation to all the four study variables, all the variables except serum albumin were positively correlated. On the other hand, CIMT and

ACR were significant ($p < 0.05$) predictable biomarkers for the assessment of cardiovascular disease in stage III and stage IV CKD patients (Table 3).

Table 1: Demographic variables of the study

Variable	Control (Mean)	Case (Mean)
Gender		
Male	68	60
Female	32	40
Age	54.07	54.54

Table 2: Chi-square paired test analysis for the study variables

Parameters	N	Mean	Mean difference	Std. Deviation	Std. Error Mean	t value	P value (2-tailed)
h-FABP	Control	100	2.3930	3.016	4.07323	.40732	-7.405
	Case	100	5.4093				
CIMT	Control	100	0.6430	0.177	.30383	.03038	-5.839
	Case	100	0.8204				
Serum albumin	Control	100	3.8610	0.65	.43263	.04326	15.024
	Case	100	3.2110				
ACR	Control	100	8.5700	434.91	176.18649	17.61865	-24.685
	Case	100	443.4800				

Table 3: Pearson's correlation analysis for the study variables

Parameters	N	Correlation	P-value
h-FABP	Control / Case	100	.057
			0.574 (in Significant)
CIMT	Control / Case	100	.218
			0.030 (Significant)
Serum albumin	Control / Case	100	-.165
			0.101 (in Significant)
ACR	Control / Case	100	.240
			0.016 (Significant)

Discussion

Patients with Chronic Kidney Disease (CKD) have a higher chance of developing cardiovascular disease, which leads to increased morbidity, mortality, and healthcare costs (CVD). To our knowledge, this is the first study to link HFABP, CIMT, SA, and ACR as an independent risk factor for the occurrence of CV disease in CKD stages III and IV patients. Most research have focused on either the general population or ESRD-hemodialysis patients in order to correlate a single parameter with CV risk, but this was the first study to look at four separate

biomarkers in CKD patients for the development of CVD at the same time. The serum levels of HFABP were elevated in stage III and IV CKD patients. An elevated HFABP level may be useful for detecting the presence of CVD events in CKD patients. In CKD patients, higher levels of the cardiac biomarker H-FABP have been linked to an elevated risk of cardiovascular illness and subsequent high mortality. Similarly, our findings reveal that H-FABP is a viable marker for all-cause and cardiovascular fatalities in the CKD population for the first time. These findings contributed to the fact that elevated H-FABP levels were significantly associated with future all-cause mortality as well as cardiovascular deaths.

Carotid intima-media thickness is a marker of atherosclerotic vascular disease that provides a full picture of all modifications to the artery walls generated by numerous cardiovascular risk factors throughout time. 4. Atherosclerosis, a key risk factor for CVD, has been demonstrated to connect with carotid intimal medial thickness (CIMT). When compared to age-matched controls, individuals with CKD had a higher CIMT. This shows that the prevalence of carotid atherosclerosis in CKD patients is much higher than in controls. The Mean value 0.82 of CIMT was significantly higher in Cases as compared to mean value 0.64 of Control group ($p < 0.05$). CIMT has been found to be higher in patients with end-stage renal disease than in healthy controls. The results of the present study were in accordance to the findings of Preston et al. [5] reported that patients with stage 3 to 4 CKD had increased CIMT compared with normotensive volunteers. Lu Xia Zhang et al. [6] in their study on stage 2-3 CKD patients (i.e., mild and moderate renal insufficiency), found significantly increased CIMT in these patients and concluded that arterial change might occur in the course of CKD earlier than previously believed. This compares favorably with findings by Zoungas et al. [7] who found significantly thickened CIMT in CKD patients compared with controls (0.89 ± 0.17 vs 0.73 ± 0.13 mm, respectively). Some other authors also reported similar findings [8, 9].

In our study population which consisted of CKD stage III and stage IV, CIMT measurement can be a good predictive marker for all-cause mortality and new CV event incidence. The Chi² test findings of the present study revealed that serum albumin is a significant risk factor for cardiovascular disease in CKD patients is highlighted by our results on 100 patients with CKD III to IV in whom low serum albumin was significantly associated with CVD. Our study also found that, there is no correlation between the increased serum albumin levels for the cardiovascular risk prediction in CKD patients. Beddhu et al. [10] showed association between serum albumin level and CVD in chronic hemodialysis patients. An association between serum albumin and cardiovascular mortality has been reported by several studies. Owen et al. [11] demonstrated that hypoalbuminemia was a strong predictor of mortality in dialysis patients. Kalantar-Zadeh et al. [12] also showed higher mortality in dialysis patients with lower albumin. Many recent studies showed serial measurement of serum albumin can even better predict chronic inflammation and clinical events [13-15]. Looking at the results of all these studies it is clear that hypoalbuminemia is adversely associated with CVD in ESRD. The results showed that, the ACR ratio was very high in CKD patients compared to control population. The albumin creatinine ratio were strongly associated with cardiovascular mortality in CKD patients. Similar results were reported by Ninomiya et al. [16] in patients with both

UACR >300 mg/g and eGFR<60 ml/min per 1.73 m² at baseline had a 3.2-fold higher risk for cardiovascular events and a 22.2-fold higher risk for renal events, compared with patients with neither of these risk factors. In conclusion, high albuminuria and low eGFR are independent risk factors for cardiovascular and renal events among patients with type 2 diabetes.

Conclusion

The increased rate of CV problems found in CKD patients is attributable to a mix of traditional risk factors as well as those that are more intimately tied to the loss of renal function (anemia, oxidative stress, inflammation, and bone mineral disorders). This implies that a prompt and accurate assessment of cardiovascular risk will enable more aggressive and focused treatment of individuals who are most in need of preventive interventions to decrease incident rates. In this paper, the authors found that, the use of established and significant laboratory biomarkers like H-FABP, CIMT, ACR, Serum albumin concentration were really proven to be assessing the CKD risk in the general population.

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