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YKL-40 as Inflammatory Marker in Reference to hs CRP in CKD Individuals with Uremic Toxicity to Diagnose the Cardio Vascular Events

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ABSTRACT

CKD one of the non-communicable diseases was forecasted to be raised in the cases, in which there is rise in uraemic toxins which may leads to secondary complication, hence we intended to study the inflammation due to uraemic toxicity. The study subjects were picked randomly. The basic information, the patient history was collected and blood samples were drawn after obtaining a written consent form. The patients were grouped into four different groups as, Group I (controls) contain normal healthy individuals free from all the systemic ailments (n=50). Group II contains individuals with CKD stage 1 &2 (n=50) Group III contains individuals with CKD stage 3, 4 & 5 (n=50). Group IV contains individuals with Hemodialysis (n=50). The study protocol was approved by the Institutional Ethics Committee. Biochemical parameters like fasting blood glucose, RFT, uric acid and inflammatory biomarkers were analyzed. Although inflammation may have protective, but in hemodialysis the inflammation does persist which can lead to development of CV events. In this regard we suggest to estimate the CRP levels along with YKL-40 to decide the doses of dialysis to reduce the inflammation as a conceptive therapy to reduce the CV events.

Key Words: *Cardiovascular Disease, Inflammation, High Sensitive - CRP, Renal Failure, Uremic Syndrome, YKL-40*

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INTRODUCTION:

Chronic kidney disease (CKD) is one of the non-communicable disease, studies of Foreman, K.J et al., 2018 has forecasted that CKD will be one of the causes for increase in years of lost life (YLL) from 2016 to 2040 which will decrease its rank from 13th to 5th place and will be becoming the major global burden [1]. Accruing evidence has indicated the pathological changes of the endothelium may play a role in the development of cardiovascular complications in CKD. Non-traditional risk factors related to CKD are associated with the incidence of cardiovascular disease, but their role in uraemicendothelial dysfunction has often been ignored [2].Inadequate dialysis during the CKD leads to accumulation of toxic substance causes fatal damages which may lead to increase in mortality ratio also. The secondary complication associated with inadequate dialysis are fluid overload, hyperkalemia, hyperphosphatemia, pruritus, restless leg syndrome, anemia, etc., can be stated as uremic toxicity [3].

Inflammation in the normal individual persist to protect the infected and damaged tissues to dilated the arteries for recruiting the WBC and plasma proteins. But the *in-vivo* inflammation in the CKD and Hemodialysis (HD) individuals might be due to dialysis membranes during HD that stimulate inflammation and activate the complement pathway. Accumulation of urotoxins *in-vivo*, secondary to intestinal dysregulation and imbalance in oxidative stress. Reduced renal function willincrease circulatory preload and presence of metabolic acidosis canalso lead to increased production of pro-inflammatory and inflammatory factors (CRP, Interleukins, etc.)can predict the prognosis of ESRD patients by effectively assessing the degree of inflammation in such patients [4, 5].

hsCRP a known marker of systemic inflammation which can predicts the risk for vascular diseases and can be a prognostic biomarker. There are many studies to report the postdialysisraise of hsCRP is associated with increased mortality among patients with hemodialysis [5, 6]. Several systemic studies have evidenced that, hsCRP may be a marker of inflammation but also mediate several key processes in the development of atherosclerosis including plaque initiation, formation, and rupture [7].

YKL-40 is a 40 kDa heparin- and mchitin-binding glycoprotein secreted by macrophages, neutrophils and cancer cells which regulates vascular endothelial growth factor. It has a role in inflammation and angiogenesis, remodelling of the extracellular matrix and fibrosis, hence it has been associated with inflammatory disorders, arteriosclerosis and endothelial dysfunction [8, 9]. The matured macrophages are major source for circulatingYKL-40 and its prime function remain mostly unknown, but thought to have a role in the proliferation of chondrocytes and fibroblasts, differentiation of macrophages, migration and reorganization of vascular endothelium, extracellular matrix remodelling, and inflammation [10].

Although several studies have put forth in predicting the CVD risk in CKD and hemodialysis individuals but was not appropriate enough to predict the risk for CVD [11]. The hsCRP being the marker of inflammation but has not highly specific to inflammation in CKD to diagnose the CVD risk. Hence, we have taken up this study to inflammatory risk in CKD and HD individuals to assess the CVD risk using YKL-40. Very few studies were reported across the world and ethnically with Indian population, we were first to report the YKL-40 levels in CKD and HD individuals to study the risk for CVD.

MATERIALS AND METHODS:

The subjects for the study have recruited the individuals who were attending to the tertiary hospital for health checkup and follow-up. These subjects were picked randomly. The basic information, the patient history was collected and blood samples were drawn after obtaining a written consent form. The study protocol was approved by the Institutional Ethics Committee.

Based on the patients age and risk factors the patients were grouped into four different groups as follow. Group I (controls) contain normal healthy individuals free from all the systemic ailments (n=50). Group II contains individuals with CKD stage 1 & 2(n=50) Group III contains individuals with CKD stage 3, 4 & 5(n=50). Group IV contains individuals with Hemodialysis(n=50). The patients with CKD were staged and grouped based on the eGFR values obtained from the eGFR calculator designed by the national kidney foundation [12].

Inclusion Criteria:

Patients of different stages of CKD including those on hemodialysis for more than three months

Exclusion Criteria:

Patients undergoing haemodialysis treatment less than three months, Age <16 years, Presence of HIV or Hepatitis B/C infection, Chronic inflammatory (malignancy, liver disease) and infective conditions, Pregnant women, Unwilling patients were excluded from the study.

Sample Collection:

A total of 10 ml overnight fasting venous blood was collected. Out of that 5 ml of blood sample was taken into plain tube and allowed to clot adequately for 15 minutes and centrifuged at 3000 rpm for 10 minutes for collecting serum and 2 ml of the blood was taken into fluoride tube for collecting plasma to estimate fasting glucose.

METHODS:

Routine parameters such as height, weight, and body mass index (BMI) were recorded. Weight was measured using a beam balance, to the nearest 0.1 kg and height to the nearest centimeter, using a tape stuck to the wall. Blood pressure levels were also recorded for all the subjects using mercury sphygmomanometer.

Routine biochemical parameters like fasting blood glucose, RFT, Calcium, phosphorous, magnesium, and Uric acid were analyzed in serum using fully automated auto analyzer (Siemens DADE BEHRING - Dimension EXL-200). The electrolytes were analyzed using (Siemens QuikLYTE® Integrated Multisensor).YKL-40was estimated by Enzyme Immunosorbent Assay (ELK Biotechnology, USA). hs-CRP was estimated bythe latex turbidometry kit (Euro Synergy Bio Premium). eGFR was calculated using the calculator designed by “national kidney foundation” using the serum creatinine, age, gender [12].

Statistics was done using SPSS 20.0 software. Results were expressed as Mean±SD. P value≤0.05 was considered significant. Group comparison is done by using ANNOVA (Post-hoc Sheffe’s alpha test). Pearson correlation was used for correlating the parameters. ROC Curve analysis was done for testing sensitivity and specificity.

RESULTS:

From Table-I, the chi-square analysis results clearly indicate that males are significantly affected with renal failure than females in Group-II, III, and IV compared to control (Group-I).Anthropometric parameters like SBP were found to be significantly elevated ($p < 0.05$) among study groups when compared to Group-I. However, DBP increased significantly ($p < 0.05$) in the Groups-II and IV, but not with Group-III compared to controls. The rise in BMI was insignificant in all the study groups compared to the control group. Increased SBP indicates the hypertension among the study groups indicate role of renal failure due to hypertension(Table-II).

Table-I: Gender Distribution among the groups

Groups			Group-I	Group-II	Group-III	Group-IV	Total
Sex	Female	Count	27	16	19	13	**75**
		Percentile	13.5%	8.0%	9.5%	6.5%	**37.5.0%**
	Male	Count	23	34	31	37	**125**
		Percentile	11.5%	17.0%	15.5%	18.5%	**62.5%**
Total		Count	**50**	**50**	**50**	**50**	**200**
		Percentile	**25.0%**	**25.0%**	**25.0%**	**25.0%**	**100.0%**

Table: II Comparison of anthropometric parameters among the groups

Groups	SBP (mmHg)	DBP (mmHg)	BMI (kg/m^2)
Group-I (n=50)	120±0.00^{a}	79.40±2.39^{a}	25.48±2.07^{a}
Group-II (n=50)	127±8.14^{b,c}	85.0±7.62^{b,c}	27.0±2.73^{a}
Group-III (n=50)	126±11.22^{c,d}	82.40±5.91^{a,c}	24.27±3.26^{a}
Group-IV (n=50)	129±13.16^{d}	84.60±7.61^{c,b}	25.4±3.01^{a}

Note: Values not sharing common superscript are significant at <0.05 level

eGFR, Creatinine and uric acid was significantly increased among Group-III and IV with no change in Group-II compared to the control group. On the other hand, FBS and Urea levels were found to be significantly elevated ($p < 0.05$) among all study groups when compared to Group-I. These results clearly indicate the rise in the uraemic toxins viz. creatinine, urea and uric acid levels along with a decrease in the eGFR rate) suggestive of renal failure(Table-III).

Table-III: Comparison of eGFR, RBS, RFT, hsCRP and YKL-40 values among the groups

Groups	eGFR ml/min/1.73m^2	FBS mg/dl	Urea mg/dl	Creatinine mg/dl	Uric Acid mg/dl	hsCRP mg/L	YKL-40 pg/ml
Group-I (n=50)	105.68±6.69^{a}	95.40±9.02^{a}	22.64±0.73^{a}	0.7±0.11^{a}	4.66±0.54^{a}	1.02±0.38^{a}	45.23±8.41^{a}
Group-II (n=50)	74.00±10.8^{a}	117.96±22.97^{b}	37.04±1.04^{b}	1.15±0.12^{a}	5.07±0.78^{a}	1.47±0.52^{a}	63.15±11.84^{b}
Group-III (n=50)	22.82±7.27^{b}	137.22±49.88^{c}	59.04±2.87^{c}	3.10±0.83^{b}	6.24±1.63^{b}	5.15±1.67^{b}	70.20±15.64^{b}
Group-IV (n=50)	6.80±1.38^{b}	133.72±29.58^{b}	90.16±2.25^{d}	8.43±1.20^{c}	6.64±1.36^{b}	6.08±1.83^{c}	106.21±30.06^{c}

Note: Values not sharing common superscript are significant at <0.05 level

The inflammatory biomarkers hs-CRP found to be significantly elevated ($p < 0.05$) among Group-III, and IV compared to control (Group-I) but not with Group-IIand YKL-40was found to be significantly elevated ($p < 0.05$) among Group-II, III, and IV compared to control (Group-I)which indicates the inflammation due to renal failure(Table-III).

Table-IV: Correlation between hsCRP and YKL-40 with Creatinine

	Creatinine	hsCRP	YKL-40
Creatinine	1	0.706**	0.715**
Sig.	-	0.00	0.00
hsCRP	0.706**	1	0.636**
Sig.	0.00	-	0.00
YKL-40	0.715**	0.636**	1
Sig.	0.00	0.00	-
**. Correlation is significant at the 0.01 level.			

Table-V: ROC curve analysis for Inflammatory biomarkers with Creatinine

Variables	Sensitivity	Specificity	Area Under Curve	Asymptotic 95% Confidence Interval		Cut-off Value
				Lower Bound	Upper bound	
hs-CRP	0.220	0.873	0.774	0.708	0.840	6.29
YKL-40	0.160	0.753	0.561	0.483	0.639	87.4
Creatinine	0.040	0.707	0.667	0.591	0.742	4.2

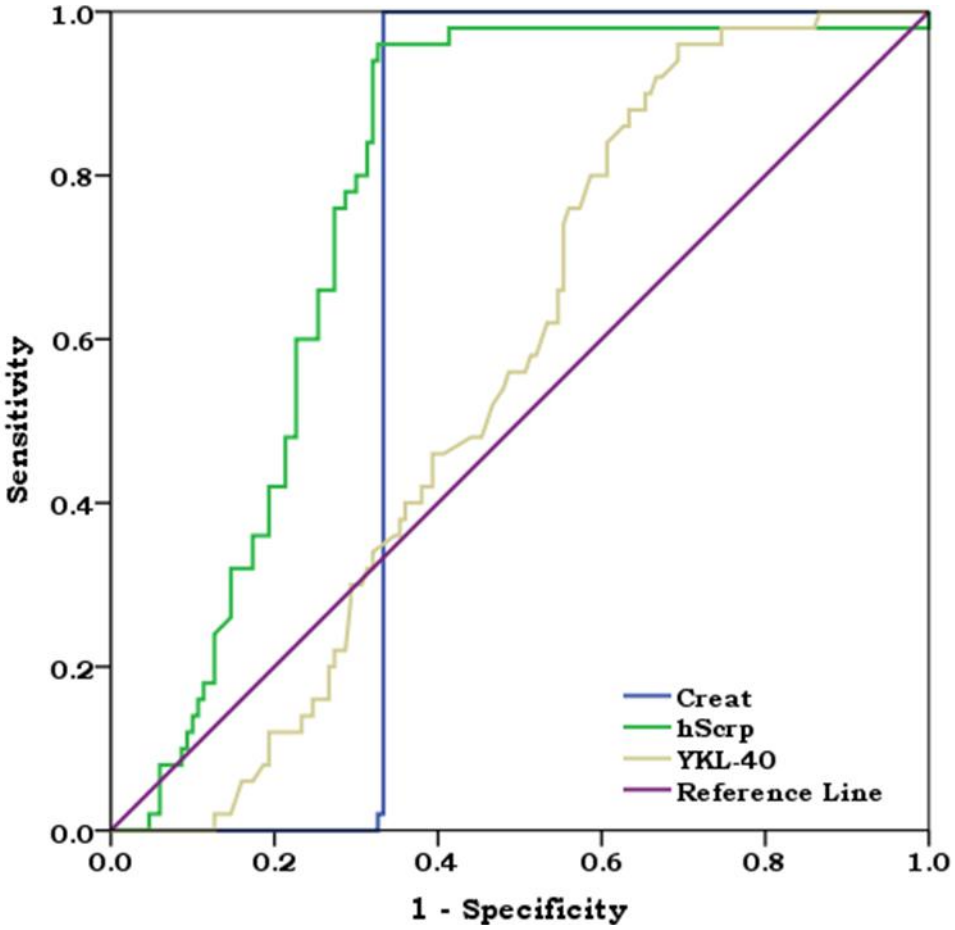

Figure-I:ROC Curve for Inflammatory biomarkers with Creatinine.

Table-IV represents the Pearson correlation between inflammatory biomarkers(hsCRP and YKL-40)and creatinine demonstrated a significant positive correlation with serum creatinine and vice versa. From the ROC Curve analysis, it is clear that hsCRP and YKL-40(Markers of inflammatory) has shown a greater area under the curve (0.774, 0.561) with high sensitivity and specificity. The results suggest that the above markers are reliable and sensitive for assessing the inflammation. The creatinine has shown 0.667 as area under curve with high sensitivity and specificity which proves to be the marker for renal failure (Table-V, Figure-I).

DISCUSSION:

As from our study we have noted a significant increase SBP and DBP in the individuals with renal failure. EunH. B et al, 2019 has done an extensive studied about the variability in both SBP and DBP under four quartiles(Q)from the means value, the Q4 represent the uncontrolled blood pressure (BP) were prone for ESRD than to the individuals under Q1 with controlled BP in long term [13]. Studies of Carmen A. P et al 2006 and Young J. H et al 2002, has shown that SBP is a strong independent risk factor that decline in kidney function among older persons with isolated systolic hypertension [14, 15].

BMI has not noted a significant change among the study groups compared with the control group. The limitation in the study was, we have not confined our study with only obese individuals with CKD. ethnic study of EnsiehM et. al 2021, say's, in men BMI and weight, is more predictive for risk of CKD than measures of abdominal obesity, i.e.,waist circumference (WC), Waist:Hip ratio (WHR) and Waist circumference: Height ratio (WHtR) and in women Body fat percentage (BF%), WHtR and WC were more predictive [16].So, obesity doesn't lead to CKD, but obesity in CKD leads

to secondary complication like inflammation leads to release to adipokines and also leads to glomerulopathy which further decline the GFR values [17].

Progressive loss of kidney function is accompanied by the retention of plenty of metabolitesin the blood and associated with fluid, electrolyte, and hormone imbalances and metabolic aberrations leading to uremic syndrome which accelerated non-traditional risk factors that include chronic inflammation, oxidative stress, sarcopenia, disordered mineral metabolism and deficiency of endogenous calcification inhibitors. The term uremia, which literally means urine in the blood[18,19]. CKD progression contributes to the oxidative stress produced by intracellular uremictoxins, leading to inflammation and tissue destruction and endothelial dysfunction/damage, which in turn contributes to the pathogenesis of cardiovascular diseases[20].

As the CKD progress to the next stages, in our study we have noted the gradual accumulation of excretory products like urea, creatinine, uric acid which indicates the uremic toxicity index and their rise has shown significant positive correlation to the inflammatory biomarkers like hsCRP and YKL-40 indicating the uremic toxicity triggering the inflammatory response in CKD. The severity is more observed in hemodialysis individuals. The ROC curve analysis has shown a greater area under curve with a good sensitivity and specificity values, in reference to the standard biomarker (creatinine) for CKD, which can able to detect the inflammation in CKD.

In our study we found elevated hsCRPin both the CKD and hemodialysis cases indicates the role of inflammation. As the uremic toxicity induces the inflammation in the endothelial cells of the artery. According to the US Centre for Disease Control and Prevention (CDC) and the American Heart Association (AHA) has given the guidelines to assess the inflammatory risk for CVD using the hsCRP, accordingly they indicated low risk for CVDifhs-CRP <1mg/L, average risk as 1 to 3mg/L, and high risk as>3mg/L [21]. Compared to this in our study group we have noted average risk in CKD individuals and high risk for hemodialysis individuals indicates in-milieu inflammatory risk for the development of CVD. How ever there are numerous to report the same.

This is the first study to report the estimation of YKL-40 in CKD to assess the risk for developing CVD in our ethnic population by considered it as a surrogate biomarkerand hsCRPas lessspecific biomarker for inflammation in these individuals.The values above the baseline levels of YKL-40 has noted a more significant raise in hemodialysis group than to CKD groups when compared to control group and the Pearson correlation noted a significant correlation with the hsCRP and creatinine also. The ROC analysis also has shown grater area under the curve but not as much as hsCRP, which proves YKL-40 as a marker of inflammation in CKD and hemodialysis individuals in assessing the risk for developing CVD. It's being a marker of poor prognosis in several cancers, the marker has been linked to atherosclerosis, rheumatologic diseases, arterial stiffness, stroke, and mortality in type 2 diabetes [22].

Vega A et al, 2019 in their study has observed that baseline chronic inflammation with the loss of renal function, and increased YKL-40.The serum YKL-40 can be removed during the dialysis in hemodialysis individuals. In addition to this they have noted the twofold raise in the serum YKL-40 after to dialysis. YKL-40 concentration increases due to inflammation and is associated with the CV events [9]. Indeed, an association between low lean tissue and CV events has been observed in patients with advanced CKD not in dialysis [23]. Gül S K et. al 2019, has stated that, activated macrophages obtained from early atherosclerotic lesions have been shown to express very high levels of YKL-40, which can be associated with cardiovascular disease and mortality [10].Which indicates the inflammatory response in atherosclerosis due to uremic toxicity.

According to the study of Jakob S et al, 2020 has found that YKL- 40 has been associated with development of hyperlipidemia and ischemic stroke in their study with larger sample size from the general population and has identified itas risk marker in both atherosclerotic development and CVD outcome prediction in patients with acute coronary syndrome and stable coronary artery disease (CAD) [24].

YKL-40, is related to inflammatory response, and like hsCRP, is not disease specific. Higher dialysis doses will lower the serum YKL-40 levels and the outcomes of will guide in regards to conceptive therapies [9]. Since atherosclerosis has an inflammatory component, it is unsurprising that YKL-40 could be used as a biomarker for identifying the early stages of this disease. Additionally, increased YKL-40 levels have been suggested to serve as a marker of renal function and composite renal outcome towards the events of CVD [25].Hence, we conclude that in conjunction with hsCRP levels, YKL-40 estimation can be done to assess the CV events especially in the individuals with hemodialysis individuals towards the conceptive therapies.

Ethics approval and consent to participate:

The study protocol was approved by the Institutional ethics committee of Malla Reddy medical college for women (Malla Reddy Narayana Multispeciality hospital), Hyderabad. With Reference No: MRMCWIEC/AP/85/2022, dated 12-04-2022.

Conflicts of Interest: The author(s) doesn't have any conflicts to declare.

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