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Original Article

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Uremic Toxins and Endothelial Dysfunction in CKD and Hemodialysis Individuals Increasing the Mortality towards CVD

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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 uremic toxins which may leads to secondary complication, hence we intended to study the endothelial dysfunction due to uremic 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 endothelial dysfunction markers were analysed. Multiple risk factors of CVD may influence the rise of ADMA and E-selectin. Our study confined to only CKD individuals has noted increased ADMA and E-selectin is due to the endothelial dysfunction which may influence CKD in development of vascular injury causes increase in mortality rate in these individuals by developing the CVD.

Key Words: *Asymmetric Dimethyl Arginine, Endothelial Dysfunction, E-Selectin, Renal Failure, Uremic Syndrome*

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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 uremicendothelial dysfunction has often been ignored [2]. With the progressive loss of kidney function there will be accumulation of uremic toxin/metabolites due to decreased renal clearance and rise in their production, among them arelow MW (<500Da) water soluble substances (asymmetric and symmetric dimethylarginine (ADMA and SDMA), oxalate, trimethylamineN-oxide (TMAO), polyamines, urea, and uric acid leading to uremia [3].

ADMA being a non-standardamino acid, free water soluble endogenous competitive inhibitor of nitrous oxide synthase (NOS). NOS usesL-arginine as a substrate to generate nitrous oxide (NO) and L-citrulline. ADMA is synthesized during post-translational methylation of arginine in proteins by protein arginine methyltransferases. Free ADMA is released upon proteolysis of ADMA-incorporated proteins. Circulating levels of ADMA are elevated in patients with CKD, even before alterations in GFR. About 20% of ADMA is excreted in the urine and increased concentration is due to the decreased excretion which will upregulate the NOX4 expression generating reactive oxygen species and activation of fibroblasts and decreased renal peritubular capillaries and increased renalfibrosis [4].

Uric acid (UA) also considered as one of the water soluble uremic metabolite formed due to degradation of purine. Decreased excretion of UA occurs in CKD with stage 4-5. Increased intracellular UA levels with subsequent ROS

formation was also shown to increase endoplasmic reticulum stress and apoptosis indicated by increased levels of caspase-12which results in a decreased NOS activity and reduced NO production [5].

Traditional risk factors like hypertension, diabetes and hyperlipidemia fully can't explain the risk of CVD in patients with CKD. Accumulation of uremic toxins also play a vital role in CKD-associated endothelial dysfunction [6].

In the light of above, we aimed to evaluate potential relationships between the plasmaconcentrations of uraemic toxins, endothelial dysfunction biomarkers (ADMA and E-Selectin) along with evaluating parameters of kidney function, such as eGFR to understand the relationship of rise in uraemic toxin leading to endothelial dysfunction.

MATERIALS AND METHODS

The subjects for the study were recruited, who attended to the tertiary hospital for health checkup. 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 based on their 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 [7].

Inclusion Criteria:

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

Exclusion Criteria:

Patients undergoing hemodialysis 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).ADMA and E-Selectin was estimated by Enzyme Immunosorbent Assay (ELK Biotechnology, USA and Ray-Biotech, USA) respectively. eGFR was calculated using the calculator designed by "national kidney foundation" using the serum creatinine, age, gender [7].

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). Endothelial dysfunction markers like ADMA and E-Selectin were found to be significantly elevated ($p < 0.05$) among Group-II, III, and IV compared to control (Group-I) which indicates the endothelial dysfunction due to renal failure (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%**
	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%**

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.

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

TableIII: Comparison of eGFR, RBS, RFT, ADMA and E-Selectin values among the groups

Groups	eGFR ml/min/1.73m^2	FBS mg/dl	Urea mg/dl	Creatinine mg/dl	Uric Acid mg/dl	ADMA ng/ml	E-Selectin ng/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}	191.58±27.81^{a}	39.29±5.15^{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}	271.60±26.79^{b}	51.68±2.86^{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}	352.76±19.17^{c}	59.79±4.43^{c}
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}	438.84±34.09^{d}	69.68±3.82^{d}

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

Increased SBP indicates the hypertension among the study groups indicate role of renal failure due to hypertension 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 uremic toxins viz. creatinine, urea and uric acid levels along with a decrease in the eGFR rate) suggestive of renal failure (Table-III).

Table-IV represents the Pearson correlation between Endothelial dysfunction markers and creatinine in all individual study groups. The ADMA and E-Selectin markers demonstrated a significant positive correlation with serum creatinine and vice versa.

TableIV: Correlation between ADMA and E-Selectin with Creatinine

	Creatinine	ADMA	E-Selectin
Creatinine	1	0.883**	0.828**
Sig.	-	0.00	0.00
ADMA	0.883**	1	0.964**
Sig.	0.00	-	0.00
E-Selectin	0.828**	0.964**	1
Sig.	0.00	0.00	-
**. Correlation is significant at the 0.01 level.			

TableV: ROC curve analysis for Endothelial dysfunction biomarkers with Creatinine

Variables	Sensitivity	Specificity	Area Under Curve	Asymptotic 95% Confidence Interval		Cut-off Value
				Lower bound	Upper bound	
ADMA	0.040	0.720	0.667	0.592	0.742	384
E-Selectin	0.060	0.800	0.659	0.585	0.733	66.8
Creatinine	0.040	0.707	0.667	0.591	0.742	4.2

From the ROC Curve analysis, it is clear that ADMA and E-Selectin (Markers of Endothelial dysfunction) has shown a greater area under the curve (0.667, 0.659) with high sensitivity and specificity. The results suggest that the above markers are reliable and sensitive for assessing the endothelial dysfunction. 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).

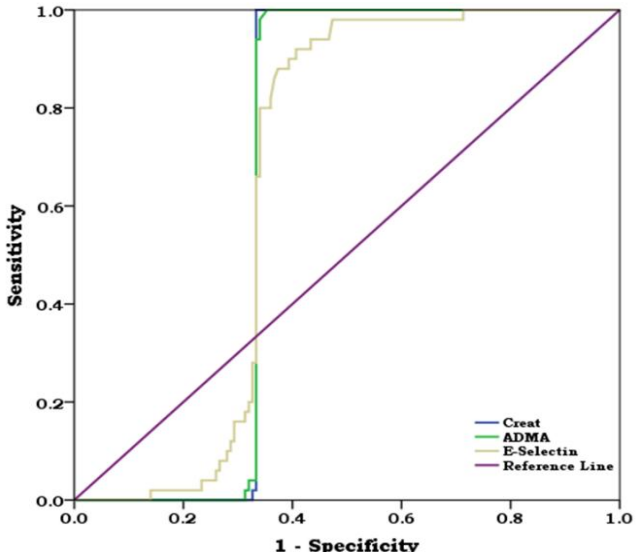

Figure I: ROC Curve for Endothelial dysfunction biomarkers with Creatinine.

DISCUSSION

As from our study we have noted a significant increase SBP and DBP in the individuals with renal failure.Eun H. B et al, 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 [8]. Studies of Carmen A. P et al and Young J. H et al., has shown that SBP is a strong independent risk factor that decline in kidney function among older persons with isolated systolic hypertension [9, 10].

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 Ensieh M.et. al, 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 [11].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 [12].

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[13, 14].With progression of CKD contributesto 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[15].

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 endothelial dysfunction biomarkers like ADMA and E-Selectin indicating the uremic toxicity damaging the endothelium and 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 endothelial dysfunction in CKD.

It is known that ADMA being the competitive inhibitor of nitric oxide synthase (NOS) which decrease the production of nitric oxide (NO) which reduced endothelium-dependent vasorelaxation, increase the arterial stiffness, vascular resistance and blood pressure [16, 17]. Study carried by Mengjie.H et al, has narrated the same and further explained, uremic toxins trigger the mitochondrial reactive oxygen species (ROS), which collectively enhances the pathophysiologicalmechanism in uremic toxins.

According to a previous study of endothelial dysfunctionis due to Mitochondrial fission mainly modulated by Drp1, where they observed altered mitochondrialmorphology, reduced network, and increased Drp1 protein expression and ROS production in endothelial cells [15]. Jourde-Chiche N et al, has also explained the endothelial dysfunction in CKD by studying the endothelial progenitor cells (EPC) circulation in correlation to the uremic toxins in hemodialysis. Uremic toxins have deleterious role on progenitor cells in cellular differentiation in early stages. Where they found myeloid EPC number was correlated positively with the vascular injury markers, and has proved that vascular lesions could stimulate progenitor cells mobilization, even in context of reduced EPC induced by CKD and concluded role of uremic toxins and vascular injury that effects the EPC neology in CKD [18].

E-Selectin being the vascular cell adhesion molecule is a marker of vascular endothelial dysfunction was expressed in in higher concentration as there is a progression in CKD stages to hemodialysis which may leads changes in vascular functions. There are few studies to report the role of E-selectin in CKD individuals leading to the CVD progression. Goligorsky MS et al, has noted the same, where they have reported increase is due to increased endothelial injury anddecreased endothelial repair [19]. A cross sectional study carried by Peter S et al, has concluded that rise in the E-selectin is due to the inflammation and malnutrition in the pre-dialysis patients which can detect the mortality of the individuals towards cardiovascular diseases [20].

However, we conclude that, numerous studies have studied the role of ADMA and E-selectin in CVD and their levels may be influenced by many risk factors like inflammation, diabetes, hypertension, smoking etc. but we have restricted our study only to the renal failure subjects only which will be limitation our study. Increased ADMA and E-selectin due to the endothelial dysfunction which may influence CKD especially hemodialysis individuals in development of vascular injury causes increase in mortality rate in these individuals by developing the CVD.

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