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RESEARCH ARTICLE

HYPERHOMOCYSTEINEMIA AS A RISK FACTOR FOR ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Background: The role of Hyperhomocysteinemia (HHomocysteine) as a risk factor for atherosclerosis in Chronic kidney disease (CKD) has gained much interest worldwide. The primary objective of the study was to ascertain the association between serum homocysteine levels and GFR.
Method: This case control study was done in stage 3,4 and 5 of CKD (cases= 63, controls =21) to assess the association between Serum homocysteine (S.Homocysteine), Serum creatinine (S.Cr), Glomerular Filtration Rate(GFR) and Carotid intima media thickness (CIMT).
Result: Statistical analysis using ANOVA and pearsons correlation revealed a significant association between S.Homo-cysteine and stages of CKD (p=0.00), S.Homocysteine and GFR (p=0.00, rsq=0.3686) S.Homocysteine and CIMT (p=0.002, rsq=0.1429) and CIMT and CKD (p=0.00). Conclusion:On the basis of these observations, it was concluded that HHomocysteine exists in CKD and that it produces atherosclerosis. Hence early screening and treatment for HHomocysteine and atherosclerosis should be done in CKD to prevent cardiovascular diseases.

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INTRODUCTION

Chronic kidney disease (CKD) is an important chronic disease that affects the world1. In stage5 chronic kidney disease (CKD) (endstage), the overall risk for stroke increases 6-fold (Puri *et al*., 2003). In patients with chronic kidney dysfunction, cardiovascular disease (CVD) is twice as common as in the general population and it advances at twice the rate (Hultberg *et al*., 1995). In an essentially non-dialysed population from the UK with a serum creatinine (SCrea) _1.7 mg/dl (_150 mmol/l), standardized mortality was increased 2-fold vs the general population in those aged >65 years, 12-fold between the ages of 50 and 64 and even 36-fold between the ages of 16 and 49. (Arnadottir *et al*., 1996) Despite the high prevalence in CKD, the traditional risk factors (old age, hypertension, diabetes mellitus, dyslipidaemia and physical inactivity) fail to entirely account for the progression of atherosclerotic diseases. Unique renal related risk factors like HHomocysteine contribute to the high risk of atherosclerosis in CKD3. CKD patients have an excess prevalence of mild to moderate HHomocysteine (85-90%) and HHomocysteine has been independently linked to Cardiovascular diseases in CKD in many recent prospective observational studies (PietroPozzoni *et al*., 2004). This study was done with the objective of finding the correlation of Homocysteinevalues withrenal function and extend of atherosclerosis in CKD patients.

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MATERIALS AND METHODS

Study design: This case control study was done at Nephrology outpatient department Government Medical college Hospital, Trivandrum, Kerala, India. The 63 cases belonged to stage 3,4 and 5 of CKD, 21 cases in each group, belonging to either gender and of 25-65 years of age. Cases with diabetic nephropathy and proteinuria of >1 gm were excluded. CKD included those patients with kidney damage for≥ 3 months with structural or functional abnormality or a GFR of < 60 ml/min/1.73m2 for >3 months with or without kidney damage. GFR of 30-59 ml/min/1.73m2 is CKD stage 3, GFR of 15-29 ml/min/1.73m2 is stage 4 and GFR of <15 ml/min/1.73m^2 is stage 5. Age and sex matched controls were selected from healthy kidney donors attending the nephrology clinic. Those donors who volunteered to participate were free of overt CKD, Coronary artery disease, Diabetes mellitus and hypertension. The protocols were submitted to and approved By Human Ethical Committee of Medical College, Thiruvananthapuram.

MATERIALS

A) Blood parameters:- Blood samples were taken for S. Homocysteine (serum homocysteine), S.creatinine, blood urea nitrogen and S.albumin estimation.

B) Glomerular Filtration Rate (GFR)

C) Carotid Intima Media Thickness (CIMT)

1. Homocysteine estimation: Homocysteine is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine assay was done using Axis Homocysteine Eia Fhomocysteine 100 Kit.

Assay principle :Axis® Homocysteine Enzyme Immunoassay (EIA) is an enzyme immunoassay for the determination of Homocysteine in blood. Protein-bound Homocysteine is reduced to free Homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay.

Estimation of Glomerular Filtration Rate (GFR): Glomerular filtration rate was calculated using the Modification of Diet in Renal study equation (MDRD) as recommended by the National Kidney Foundation Kidney Disease Outcome Quality Initiative (KDOQI). The MDRD Equation is as follows :- GFR (ml/min/1.73m 2) = 170 ×(SCr)-0.999 ×(Age)-0.176×(Serum Urea N) 0.370×(Alb) 0.318 × (0.762 if female) ×(1.180 if black).

The cases with GFR of >15 to <60 ml/min/1.73 m2 were selected for the study. Carotid Intima Media Thickness Study After taking informed consent and relevant history, high resolution After taking informed consent and relevant history, high resolution ultrasonographic examination of the common carotid arteries was carried out with 6 - 12 MHz linear probe in GE VOLUSON PRO machine. Patient was placed in supine position with neck slightly extended; head was placed away from the examination site. Intima-media complex thickness was measured at 1 - 1.5 cm proximal to the carotid bulb in longitudinal plane.

The area under study had to be free of plaque. Scanning of both side arteries was performed in anteroposterior projections and to obtain a better image, sound wave was adjusted perpendicularly to the arterial surface of the posterior wall of the vessel, yielding two parallel echogenic lines which corresponds to lumenintima and media-adventitia interfaces. The distance between the lineswas taken as the combined thickness of the intima and media (IM complex).

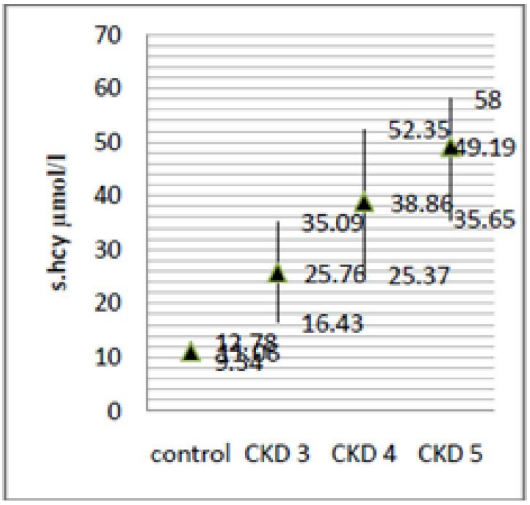

Figure 1. Serum Homocystien Values in µmols /L in Cases and Controls

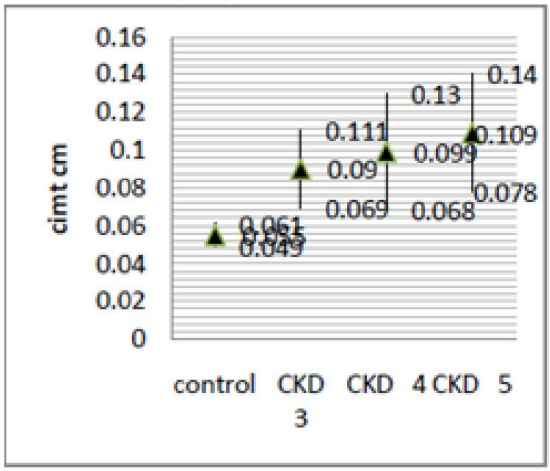

Figure 2. Carotid intima & media thickness in Cases and Controls (in Cm)

STATISTICAL ANALYSIS: Statistical analysis was done Using ANOVA and Pearson's correlation.

Table 1. Mean values of variables in each group

Group	Stage of CKD	Age (yrs)	Weight	S.Creatinine (µmol/L)	GFR Ml/min/1.73m^2	S.Homocysteine (µmol/L)	CIMT (Cm)
Controls	Mean SD	46.00 10.43	70.43 6.98	79.96 10.26	86.67 13.70	11.06 1.72	.055 .006
CKD 3	Mean SD	41.57 10.98	63.43 13.39	168.80 30.73	39.29 9.59	25.76 9.33	.090 .021
CKD 4	Mean SD	45.00 11.70	51.38 6.54	287.09 58.35	21.71 5.16	38.86 13.49	.099 .031
CKD 5	Mean SD	45.67 13.89	52.52 6.87	744.66 319.21	8.57 3.59	49.19 13.54	.109 .031

Table 2. Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	7.300E-02	.009		8.085	.000
	S.HOMOCYSTEINE	7.014E-04	.000	.378	3.189	.002

a. Dependent Variable: CIMT

RESULTS AND DISCUSSION

Serum Homocysteine and CKD: In this case control study , the mean value of the S. Homocysteine in the controls is found to be 11.06+1.72 µmol/L. The S. Homocysteine value increases significantly across the stages of CKD –25.76+9.33 µmol/L in stage 3, 38.86 +13.49 µmol/L in stage 4 and 49.19 + 13.54 µmol/L in stage 5 (Table 1, Fig 1). ANOVA of S. Homocysteine between cases and controls as well as between the groups of cases is significant at p value = 0.000. The above results are consistent with the previous studies, which date from 19/7/94, that hyperhomocysteinemia is associated with CKD. Arnadottir *et al*. (1995), Chauveau *et al*. (1996), Hultberg *et al*. (Hultberg *et al*., 1993), Cleveland clinic studies (Robinson *et al*., 1996, Litaoruan *et al*. (2009), Samuelsson *et al*10 and Wilcken *et al*. (2003) consistently found a significant association between hyperhomocysteinemia and CKD. Various reasons has been cited in many review articles for this significant association.

Arteriovenous studies in normal rat and human kidneys show that Homocysteine is normally filtered, reabsorbed and metabolized by the kidney. Hence only minimal levels of Homocysteine is normally excreted in urine. In CKD, the metabolism and filtration is altered, leading to HHomocysteine (Allon *et al*., 2001). Retained uremic toxins inhibit extrarenal Homocysteine metabolism by inducing transsulfuration defects 12.

Genetic polymorphisms of C677T also determine the Homocysteine levels in CKD. Certain studies report that HHomocysteine is the cause rather than the consequence of CKD (Coen *et al*., 2001).

Serum Homocysteine and Glomerular Filtration Rate

In this study, serum homocysteine shows a significant negative linear relation with GFR (p=0.000, rsq=0.3686). This relation is consistent with the previous studies from various parts of the world. This significant relation suggests that kidney plays an important role in plasma Homocysteine handling. GFR values estimated from Serum creatinine or calculated creatinine clearance is consistently and inversely correlated with plasma Homocysteine levels. Certain studies suggest that the relation between S.Homocysteine and GFR is because of creatinine, from which GFR values are estimated. But,studies which estimated GFR by other Method (serum creatinine, Creatinine clearance, plasma iohexol clearance, 51 Cr-EDTA clearanceor plasma Cystatin C) have shown that declining renal function is associated with high Homo-cysteine levels. This inverse relation extends from normal to End stage renal disease and to hyperfiltrating diabetic nephropathy (Allon N Friedman *et al*., 2011).

Certain studies suggest that the relation between S.Homocysteine and GFR is because of creatinine, from which GFR values are estimated. But, studies which estimated GFR by other Method (serum creatinine, Creatinine clearance, plasma iohexol clearance, 51 Cr –EDTA clearanceor plasma Cystatin C) have shown that declining renal Function is associated with high Homocysteinelevels. This inverse relation extends from normal to End stage renal disease and to hyperfiltrating diabetic nephropathy (Allon N Friedman *et al*., 2011). Some studies suggest that HHomocysteine causes intrarenal arterio-sclerosis or arterial hyalinosis, resulting in reduced renal perfusion pressure. This leads to focal or global glomerulosclerosis, tubular atrophy and interstitial fibrosis . This can also be the reason for a negative relation of S.Homocysteine with GFR11 (Coen Van Guldener *et al*., 2001).

Carotid Intima Media Thickness And CKD

In the present study, the mean CIMT increase with progress in Stages of CKD. In the controls the mean value is 0 .055 cm+0.006, 0.090+0.021 cm in stage 3, 0 .099 + 0.031cm in stage 4, 0.109 +0.031 cm in stage 5 (Table 1, Fig. 2). ANOVA shows significant association between CIMT and CKD. (p=. 000). This association is consistent with previous studies done by Baptista *et al*. (2008), Benedetto *et al*. (2008), Bevc *et al*. (2006), Kumar *et al*. (2009), Ryuichi *et al*. (2008) and Zoungas *et al*. (2009). The main reasons for the increased incidence of atherosclerosis in CKD are dyslipidaemia, oxidative stress, Hyperhomocysteinemia and raised markers of inflammation (CRP, fibrinogen and cytokines) (Iliou *et al*., 2005). Serum Homocysteine And Atherosclerosis. In this study, Carotid intima media thickness, a marker of atherosclerosis, shows a significant positive linear relation with homocysteine values (p= 0.002, rsq=0.1429). This relation is consistent with most of the previous studies. Several mechanisms have been postulated by which Homocysteine might cause atherosclerosis and atherothrombosis: Homocysteine metabolism generates reactive superoxide radicals which cause endothelial injury.

It promotes vascular smooth muscle proliferation by stimulation of the mitogen –activated protein kinase signal transduction pathway and DNA synthesis. Homocysteine promotes adhesion between neutrophil and endothelial cells. Homocysteine oxidizes LDL and promotes the cellular uptake of modified LDL (Ryuichi Kawamoto *et al*., 2008). Homocysteine induces the expression of TDAG51 which increases apoptosis and the risk of rupture of athero-sclerotic lesions by decreasing its stability 20.

Conclusion

Mild to moderate hyperhomocysteinemia exists in CKD and Serum homocysteine shows a significant negative correlation with GFR.Carotid intima media thickness is significantly elevated in the groups of CKD and it shows a significant positive relation with S.Homocysteine. This suggests that HHomocysteine in CKD is atherosclerotic and early detection of atherosclerosis can be done with carotid intima media thickness study .Along with the screening and treatment of

other risk factors of atherosclerosis in CKD, hyperhomocysteinemia should also treated. Further experimental, biochemical, genetic and prospective follow up studies are required for understanding the pathophysiology and consequences of hyperhomocysteinemia in CKD.

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