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CORRELATION OF SERUM HOMOCYSTEINE LEVELS WITH CAROTID ATHEROSCLEROSIS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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ABSTRACT

INTRODUCTION: Chronic kidney disease (CKD) is associated with significantly increased morbidity and mortality. Hyperhomocysteinemia (>15 µmol/l) is one of the such risk factors2 for CVD in CKD patients. Hyperhomocysteinemia is observed in at least 85% patients with ESRD3. With this background in mind, the present study was designed to assess severity of hyperhomocysteinemia in advanced stages of CKD and find a correlation between Hcy and carotid intima media thickness in Indian population. To assess serum homocysteine levels in patients with stage 3, 4 and 5 CKD and compare with healthy controls. To assess correlation of serum homocysteine levels with CIMT as determined by high resolution carotid ultrasound.

METHODS: A total of 66 subjects aged 20-60 years were recruited and divided into the following groups . Group A – healthy controls (n=22) Group B – stage 3 and 4 CKD (n=22) Group C – stage 5 CKD (n=22) on the basis of history, physical examination, hematological and biochemical investigations (blood urea, serum creatinine and GFR values), attending Medical OPD, Nephrology clinic, Medical Emergency or admitted in Medical Wards not meeting any of the exclusion criteria were included in the study.

RESULTS: In our study mean serum homocysteine levels in group A was 9.1±3.37 µmol/l) with a median 8.16 µmol/l and a range of 4.8-18.5 µmol/l, in group B mean homocysteine level was 22.0±3.10 µmol/l with a median 22.21 µmol/l and a range of 16.2-28.0 µmol/l. in group C pre dialysis mean homocysteine level was 29.6±4.90 µmol/l with a median 29.68 µmol/l and range 21.0-37.7 µmol/l while in group C post dialysis mean homocysteine level was 23.0±4.20 µmol/l with a Median 21.73 and range 17.2-30.1 µmol/l. Thus, homocysteine levels in CKD patients were significantly higher in (>4 times) than controls (p <0.000). In our study we tried to find out correlation between homocysteine and carotid intima-media thickness in various study groups. We found that serum homocysteine levels were higher in patients of CKD and with increase in severity of renal disease, CIMT also showed an increase. There was positive correlation between serum homocysteine level and CIMT (r=0.311) and this correlation was found to be statistically significant (p<0.001).

CONCLUSION:

- Mean serum homocysteine levels in CKD patients were significantly higher in (>4 times) than controls (p <0.000)
- There was positive correlation between serum homocysteine level and CIMT (r=0.311) and this correlation was found to be statistically significant (p<0.001).

INTRODUCTION:

Chronic kidney disease (CKD) is associated with significantly increased morbidity and mortality. Among the various causes, cardiovascular diseases (CVD) are the major contributors toward this. As per report of United state Rural Data System (USRDS), in patients of end stage renal disease (ESRD), approximately half of deaths were contributed by CVD[1]

Hyperhomocysteinemia (>15 µmol/l) is one of the such risk factors[2] which is observed in at least 85% patients with ESRD[3]. Several studies have reported that homocysteine levels rise with increasing renal dysfunction[4-6]. Observational studies in patients with CKD have suggested a causal association between hyperhomocysteinemia and cardiovascular disease[7,8]. However, in a multicentric randomized controlled trial in CRF patients, no correlation between homocysteine levels and carotid intima media thickness was found[14]. Carotid intima media thickness (CIMT) can be used as a surrogate marker of carotid atherosclerosis in patients of chronic kidney disease. With this background in mind, the present study was designed to assess severity of hyperhomocysteinemia in advanced stages of CKD and to evaluate the effect of hemodialysis on Hcy level and find a correlation between Hcy and carotid intima media thickness in Indian population. To assess serum homocysteine levels in patients with stage 3, 4 and 5 CKD and compare with healthy controls. To assess correlation of serum homocysteine levels with CIMT as determined by high resolution carotid ultrasound.

METHODS:

This case-control prospective study was carried out in the Departments of Medicine, Pathology and Radiology at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi during November 2009 to April 2011. A total of 66 subjects aged 20-60 years were recruited and divided into the following groups. Group A – healthy controls (n=22) Group B – stage 3 and 4 CKD (n=22) Group C – stage 5 CKD (n=22). In group C, serum homocysteine levels were studied just before and after a single session of hemodialysis. Twenty two patients of stage 3 and 4 CKD and another 22 patients with stage 5 CKD on the basis of history, physical examination, hematological and biochemical investigations (blood urea, serum creatinine and GFR values), attending Medical OPD, Nephrology clinic, Medical Emergency or admitted in Medical Wards not meeting any of the exclusion criteria were included in the study. Patient with megaloblastic anemia, hypothyroidism, psoriasis, chronic alcoholism.

Glomerular filration rate (GFR) was calculated using the formula for Modification of Diet in Renal Disease (MDRD) study as stated below[142].

Estimated GFR (ml/min/1.73 m^2) = 170 x ($S.Cr)^{-0.999}$ x $(age)^{-0.176}$ x $(BUN)^{-0.170}$ x (S. albumin)$^{+0.318}$ x (0.762 if female) x (1.180 if black)

The control group was comprise of age and sex matched 22 healthy subjects not meeting any of exclusion criteria were included in the study

SERUM HOMOCYSTEINE ESTIMATION :
For homocysteine estimation after at least 12 hours of overnight fasting 2ml blood samples was collected in EDTA vials. The samples were centrifuged immediately for about 10-15 minute and the aliquot of plasma stored at -20.c. plasma homocysteine concentrations were determined by AXIS Homocysteine enzyme immunoassay (EIA). Protein –bound Hcy is reduced to free Hcy and enzymatically converted to s-adenosyl-L-homocysteine (SAH) in a separate procedure prior to immunoassay. The enzyme is specific for the L-form of homocysteine, which is only form present in blood.

MEASUREMENT OF CAROTID INTIMA MEDIA THICKNESS:
Carotid intima media thickness will be measured on both side 1.5 cm proximal to the carotid bifurcation in common carotid artery bilaterally in plaque free region. However, the presence of any plaque, ulceration or calcification will be duly noted. Measurement will be done on Grayscale longitudinal image of the artery on a high-resolution image taken with the help of 7.5-10 MHz transducer on HDI 5000 or HDI 1500 USG machine (ATL Phillips).

STATISTICAL ANALYSIS:
Serum homocysteine (Hcy) levels in both patients groups were compared with levels those in healthy controls by using one-way ANOVA test. In patients with stage 5 CKD, correlation between Hcy and CIMT were done by using correlation and regression analysis.

RESULTS:
DEMOGRAPHIC PROFILE:
The study included 66 subjects distributed in three groups.

Group A:
Included 22 healthy subjects with mean age 41.6±10.9 years, including 12 (54.5%) males and 10 (45.5%) females.

Group B:
Included 22 patients with chronic kidney disease (CKD) stage 3 and 4 with mean age 45.3±10.1 years, including 11 (50%) males and 11 (50%) females.

Group C:
Included 22 patients with CKD stage 5 with mean age 40.9±12.0 years, including 13 (59.0%) males and 9 (41.0%) females.

Demographic profile of the study population is shown in Table 1

Table 1: Demographic profile of the study population

Variable	Group A (Healthy controls)	Group B (Stage 3 & 4 CKD)	Group C (Stage 5 CKD)	p value
Age (yrs.)	41.6±10.9 (21-58)	45.3±10.1 (28-60)	40.9±12.0 (21-60)	A vs B=0.517 A vs C=0.974 B vs C=0.391
Sex				
Males	12 (54.5%)	11 (50%)	13 (59.1%)	NA
Females	10 (45.5%)	11 (50%)	9 (40.9%)	NA

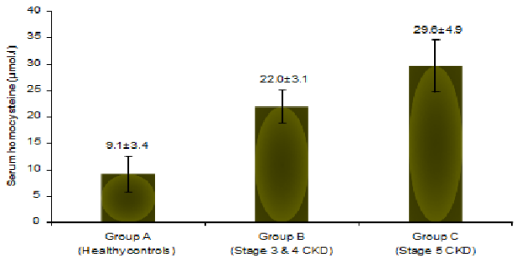
p value significant at<0.05 and highly significant at<0.001
Age expressed as mean±SD. Range is mentioned in parenthesis

SERUM HOMOCYSTEINE LEVELS IN STUDY POPULATION:
In our study (Table 2 & Fig 1) mean serum homocysteine levels in group A was 9.1±3.37 µmol/l) with a Median 8.16 µmol/l and a range of 4.8-18.5 µmol/l,in group B Mean homocysteine level was 22.0±3.10 µmol/l with a Median 22.21 µmol/l and a range of 16.2-28.0 µmol/l. In group C mean homocysteine level was 29.6±4.90 µmol/l with a Median 29.68 µmol/l and range 21.0-37.7 µmol/l.Thus homocysteine levels in CKD patients were significantly higher in (>4 times) than controls (p <0.000).

Table 2: Serum homocysteine levels in study groups (µmol/l)

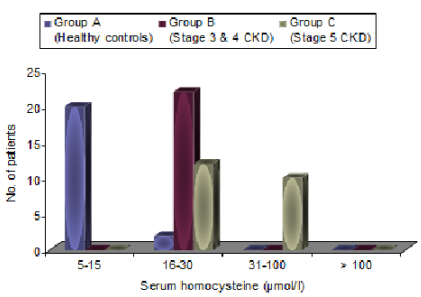
	Group A (Healthy controls)	Group B (Stage 3 & 4 CKD)	Group C (Stage 5 CKD)		p value
			Pre-dialysis	Post-dialysis	
Mean±SD	9.1±3.4	22.0±3.1	29.6±4.9	23.0±4.2	A vs B <0.001 A vs C (pre) <0.001 B vs C (pre) <0.001 C pre- vs post-dialysis <0.001
Median	8.2	22.2	29.7	21.7	-
Range	4.8-18.5	16.2-28.0	21.0-37.7	17.2-30.1	-

Fig 1: Serum homocysteine levels in various study groups:



Frequency distribution of serum homocysteine:
Fig.2 show frequency distribution of homocysteine in CKD patients and control groups. As evident from the data, all 22(100%) CKD stage 3,4 and5 patients had serum homocysteine levels above 15 umol/l as compared to only 2 out of 22 (9.09%) controls. On further analysis of the data it was found that markedly raised homocysteine levels above 30 mg/dL were present in 10 out of 22 (45.45%) patients of CKD stage 5, whereas none of the controls had values above this level.

Fig. 2: Frequency distribution of homocysteine



CAROTID INTIMA-MEDIA THICKNESS (CIMT)

Right and left CIMT values were added and averaged to give average CIMT for each subject. The average CIMT of all the subjects belonging to each group was added respectively to find out mean and SD of average CIMT in various groups to give 'mean CIMT'. Mean CIMT showed a progressive increase with declining renal function, it was 0.042±0.012 cm in group A (controls), 0.061±0.011 cm in group B (stage 3 & 4 CKD) and 0.060±0.009 cm in group C (stage 5 CKD) (Table 3). The mean CIMT in group B and group C was significantly higher than that in group A (p<0.001). Although patients of group B had a higher mean CIMT than that of group C, the difference was not statistically significant.

Table 3: CIMT profile of study subjects

Variable	Group A (healthy controls)	Group B (stage 3 & 4 CKD)	Group C (stage 5 CKD)	P value
Right CIMT (cm)	0.042±0.012	0.060±0.013	0.060±0.011	A vs B <0.001 A vs C <0.001 B vs C 0.991
Left CIMT (cm)	0.042±0.012	0.062±0.011	0.060±0.008	
Average CIMT (cm)	0.042±0.012	0.061±0.011	0.060±0.009	

Calcification and plaques in carotid arteries

Calcifications were seen in 1 (4.5%) patient of group A (controls), 3 (13%) patients of group B (stage 3 & 4 CKD) and 6 (27.3%) patients of groups C (stage 5 CKD). Group B and group C had a higher percentage of patients with carotid artery calcification as compared to group A. Also, patients in group C had a higher percentage of calcification than in group B (Table 4). This frequency distribution of calcification was found to be statistically significant along groups.

Vascular plaques were found in 1 (4.5%) patient of group A, 3 (13%) patients of group B and 4 (18.2%) patients of group C. Group B and C had a higher percentage of patients with plaques as compared to group A. Also, patients in group C had a higher percentage of plaques than in group B (Table 4). The frequency distribution of plaques was also found to be statistically significant along groups.

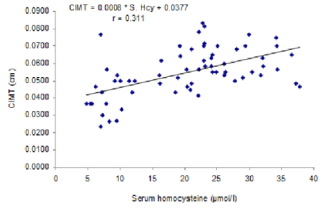
Table 4: Carotid artery plaques and calcifications in various study groups

Variable	Plaques	Calcifications
Group A (Healthy control)	1 (4.5%)	1 (4.5%)
Group B (Stage 3 & 4 CKD patients)	3 (13.0%)	3 (13.0%)
Stage 3	1 (9.1%)	1 (9.1%)
Stage 4	2 (18.2%)	2 (18.2)
Group C (stage 5 CKD)	4 (18.2%)	6 (27.3%)

Correlation between serum homocysteine and CIMT

In our study we tried to find out correlation between homocysteine and carotid intima-media thickness in various study groups. We found that serum homocysteine levels were higher in patients of CKD and with increase in severity of renal disease, CIMT also showed an increase. As shown in Fig. 3, there was positive correlation between serum homocysteine level and CIMT (r=0.311) and this correlation was found to be statistically significant (p<0.001).

Fig.3: Correlation between serum homocysteine level and mean CIMT



DISCUSSION:

High homocysteine values in patients as compared to control group

In our study, mean homocysteine level was 9.1±3.4 µmol/l in group A (controls), 22.0±3.1 µmol/l in group B (stage 3 & 4 CKD), 29.6±4.9 µmol/l in patients of group C (stage 5 CKD). Our results are in accordance with that of Nerbass et al[9], a cross-sectional study of 66 patients with moderate to severe renal impairment and 20 healthy controls. In their study, mean serum homocysteine level in CKD patients (25.4 ±13.6 umol/l) was significantly higher than in controls (10.4±3.2 umol/l) (p<0.001). Wang et al[10] also reported significantly higher levels of Hcy in CKD patients who were on chronic hemodialysis (29.5±13.6 umol/l) than in healthy controls (13.5±4.5 umol/l, p<0.01).

Parsons et al[11] conducted a study in 197 patients with renal impairment. Patients were divided into five groups according to GFR. Mean Hcy levels in the five groups were as follows: (i) GFR less than 10 ml/min - 30.2±9.8 µmol/l; (ii) GFR 10 to 20 ml/min - 26.6±10.5 µmol/l; (iii) GFR 20 to 30 ml/min - 23.9±8.6 µmol/l; (iv) GFR 30 to 45 ml/min - 22.2±8.6 µmol/l; and (v) GFR 45 to 75 ml/min - 18.2±9.1 µmol/l as compared with healthy controls - 12.7±4.6 µmol/l. Defining hyperhomocysteinemia as Hcy levels greater than the 90th percentile of controls, the level increased with declining renal function. Fifty-eight percent of patients with GFR less than 10 ml/min had hyperhomocysteinemia, and even in patients with mild renal impairment, 20% of patients had higher levels of homocysteine.

CORRELATION BETWEEN SERUM HOMOCYSTEINE AND CIMT

In our study we tried to find out whether there is any correlation between serum Hcy concentration and carotid intima-media thickness in CKD patients. When data of all patients of CKD in group B and C was pooled and analyzed to study if there was any association between Hcy levels and CIMT, a significant positive correlation was found between the two variables, indicating that with increase in serum levels of Hcy, there was significant increase in the carotid intima media thickness reflecting higher incidence of carotid atherosclerosis (r = 0.311, p<0.001). Our results are similar to there of Shoji et al[12] who examined IMT in patients with CRF and healthy controls and found that IMT was significantly higher in CRF patients as compared to normal healthy controls. After multiple regression analysis they concluded that renal failure was an independent risk factor for increased CIMT. Similarly Leoncini et al[13] examined 358 hypertensive patients with CRF and found that patients with deteriorating renal function had higher mean value of CIMT. The mean value of CIMT in CRF patients were 0.7 mm as compared to 0.65 mm in those with normal renal function. According to the authors, with an increase in severity of renal dysfunction there is increased risk of left ventricular hypertrophy (LVH), carotid atherosclerotic plaque and increased CIMT. After correction for age, hypertension duration and severity of hypertension, it was found that carotid atherosclerosis increased by 43% for each 10 ml/min decline in creatinine clearance.

In a study conducted by Lubomirova et al[14], 56 patients with a mean creatinine clearance of 39.2±10.1 ml/min and 20 healthy controls were examined and the association of Hcy levels, classic risk factors for atherosclerosis and CIMT was evaluated. CIMT values in all examined patients were significantly higher than values in healthy controls (0.75±0.006 mm vs 0.60±0.100, with p<0.001). In their study significant predictors of higher CIMT were age (r=0.358, p<0.04), duration of hypertension (r=0.395, p=0.023), diabetes (r=0.343, p<0.02) as well as duration of CRF (r=0.324, p=0.006) and there was significant positive correlation between Hcy and CIMT (r=0.344, p<0.015). Similarly Sydor et al[15] also found significant correlation

between serum Hcy and CIMT in a study carried out on 100 patients undergoing hemodialysis. In patients with mild hyperhomocysteinemia, CIMT was 0.68±0.24 mm whereas in patients with moderate hyperhomocysteinemia, CIMT was 0.80±0.25 mm. Thus, they noted a positive correlation between level of homocysteine and CIMT. Baptista et al[16] studied 56 patients of CKD with a mean eGFR of 15.8 ml/min. CIMT was used as a dependent variable in a simple linear regression model, with various laboratory parameters as independent variables. In multiple regression analysis, homocysteine was independently correlated with CIMT (p=0.027). On the other hand, Tungkasereerak et al[17] did not find any correlation between Hcy levels and CIMT. Hence, the results of our study match with that of Lubomiroval et al[14] Baptista et al[16] and Taruangsri et al[18]. Chronic kidney disease is associated with marked risk for accelerated atherosclerosis by virtue of many factors including hyperhomocysteinemia, hypertension, dyslipidemia, chronic inflammatory state and other uremic toxins. Our study showed that chronic kidney disease (stage 3 to 5) is associated with hyperhomocysteinemia and its levels rise further with increase in severity of CKD. A single session of hemodialysis lowers the Hcy levels significantly. Further, hyperhomocysteinemia may be an important contributing factor in pathogenesis of hypertension also.

CONCLUSION:

- Mean serum homocysteine levels in CKD patients were significantly higher in (>4 times) than controls (p <0.000)
- There was positive correlation between serum homocysteine level and CIMT (r=0.311) and this correlation was found to be statistically significant (p<0.001).

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