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Original Research Article

Role of homocysteine level as risk factor in the occurrence of cardiovascular events in renal transplant recipients

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ABSTRACT

Background: Traditional risk factors like elevated homocysteine levels may not completely explain the higher CVD seen in RTRs. Identification and optimisation of modifiable risk factors may help to reduce the occurrence of CVD in such population. To study the role of homocysteine level as risk factor in the occurrence of cardiovascular events in renal transplant patients. Another objective was to evaluate the other risk factors in the occurrence of CVD in such population.
Methods: Thirty renal transplant recipients and thirty healthy controls were studied. Inclusion criteria were transplant duration >6 months and patients with chronic stable renal function over the last 3 months. Samples for fasting plasma homocysteine were collected and plasma homocysteine was then estimated. All the patients were followed up every month for 6 months and evaluated for occurrence of any cardiovascular event.
Results: The mean hornocysteine levels were found to be 27.4±7.902 µmol/L in cases and 10.86±1.98 µmol/L in controls. There was no statistically significant relationship between homocysteine levels and transplant duration, mean IMT levels, proteinuria, and presence of left ventricular hypertrophy or choice of immunosuppressive regimen. Of the 30 patients, 6 patients (20%) had evidence of cardiovascular event. In the absence of other conventional factors, age of the patient, creatinine clearance (index of graft function) and mean intima-media thickness were more closely related with cardiovascular events.
Conclusions: Plasma homocysteine failed to show as an independent risk factor for cardiovascular events. New, emerging cardiovascular risk factors (e.g. Lipoprotein (a), high sensitivity C-reactive protein, fibrinogen, tissue plasminogen activator and plasminogen activator inhibitor-1) should be studied to design effective therapy to delay the progression of atherosclerosis and prolong the life of renal transplant recipients.

Keywords: Hyperhomocysteinemia, Risk factors, Cardiovascular diseases, Homocysteine, Renal transplant

INTRODUCTION

Occurrence of cardiovascular disease (CVD) complications definitely influences the long-term survival of renal transplant recipients (RTRs). Cardiovascular disease still remains a leading hazard limiting the life expectancy in RTRs. It alone accounts for 35–50% of all-cause mortality in this unique population.[1] Occurrence of cardiovascular events in renal transplant patient is multifactorial (multiple traditional and non-traditional

factors) in origin. Elevated homocysteine levels were independently associated with CV events and mortality in stable RTRs, although its mechanism is not fully understood. There is no Indian registry and hence there is no national data on survival after renal transplantation.[2]

Recently a population-based study has shown that there is little or no association between moderate hyperhomocysteinaemia and CVD risk. Any high quality evidence like randomised clinical trial has failed to prove the efficacy of lowering the homocysteine concentration as a means of lowering the incidence of CVD.[3] This controversy has created a confusion, which need to be addressed. Researchers have long debated the extent to which homocysteine should be considered as a risk factor for cardiovascular diseases, since according to some, only 50% of CVD can be explained by "classical" risk factors, and they say that "new" risk factors could significantly boost the CVD predictive power.[4]

Traditional risk factors like elevated homocysteine levels may not completely explain the higher CVD seen in RTRs, thus we need to consider other factors which may be influencing precipitation of such cardiovascular events in order to improve morbidity and mortality in this complex population.[5,6] Identification and optimisation of modifiable risk factors may help to reduce the occurrence of cardiovascular events in renal transplant recipients. Therefore the present study was planned to analyse and ascertain the occurrence of cardiovascular events in renal transplant patients. Another objective was to evaluate the risk factors in the occurrence of cardiovascular events after renal transplantation.

METHODS

The study was conducted in the Department of Medicine, Choithram Hospital and Research Center from 2012-2013. The investigations were done in the Department of Biochemistry, and Department of Radiodiagnosis. It was planned to study thirty renal transplant recipients along with equal number (n=30) of healthy controls attending nephrology clinic or admitted in Choithram Hospital. Purposive sampling technique was adopted. Inclusion criteria were transplant duration >6 months and patients with chronic stable renal function over the last 3 months. Patients with sever hyperlipidemia (LDL cholesterol >190 mg/dl or triglycerides >500 mg/dl), Patients with severe hypoalbuminemia <2 gm%, smokers and diabetics were excluded from this study.

All patients were subjected to thorough history taking and complete physical examination. Particulars such as name, age, sex, address and contact information were noted in a pre-structured proforma. Subjects were also asked regarding the duration of transplant, donor, blood group, blood transfusion before transplantation, hemodialysis duration before transplant and any past history of cardiovascular events. Weight in kilograms and height in centimeters was recorded. Upon entering the study, hemogram, serum urea, serum creatinine, serum sodium, serum potassium, blood sugar (random), Urine routine and microscopy, lipid profile ECG, echocardiography, carotid intima media thickness was captured.

Samples for the fasting plasma homocysteine were collected. Patients were made to fast overnight and samples were collected in the morning in the EDTA vials. Samples were immediately transported to the laboratory on ice and centrifuged to obtain the plasma. All the samples were thawed together, after all the samples were collacted. The plasma homocysteine was then estimated by the ELISA method using the DIAZYME Kit.

Ultrasonographic scanning of the carotid arteries was performed in the supine position with the neck extended, using a high-frequency imaging probe (7.5 MHz), at a depth of 2 cm, as the carotid vessels are relatively superficial. Scanning of the extracranial common carotid artery in the neck was performed bilaterally according to anterior posterior projection. All measurements were made at the time of scanning with the instrument's electronic calipers. Two echogenic lines separated by a hypo-echoic or anechoic space characterize its scan pattern. The outer line corresponds to the medial adventitial border and the inner line to the luminal-intimal border. The 1MT was defined as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. At each longitudinal projection, three determinations of IMT were conducted. The 3 values of each side were averaged to get the mean IMT of each side.

All the patients were followed up every month for 6 months in the outpatient clinic and record was made of any occurrence of cardiovascular event like angina, angina equivalent, unstable angina, myocardial infarction, TIA and stroke.

The study adhered to the tenets of the Declaration of Helsinki for research in humans. Informed consent was obtained from study subjects after discussing advantages and risks. Permission of Institutional ethics committee (IEC) was sought before the commencement of the study. After compilation of the collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 20 (IBM, Chicago, USA). Results are expressed as Mean ± SD. The student t-test was used for comparing differences between groups. Categorical data was analysed was using Chi-square test or Fisher's exact t test. Spearman Pearson tests was used to evaluate the relationship between variables. Statistical test results having a probability of <0.05 were considered statistically significant.

RESULTS

The mean age of the transplant patients in the present study was 32.8±9.84 years with mean transplant duration of 37.73±32.65 months. The mean creatinine clearance in the study group was 59.52±13.97 ml/min. All the patients were on antihypertensive treatment. The mean systolic

BP was 148.07±15.371 mmHg. The mean diastolic BP was 87.93±8.509 mmHg. The mean BMI was found to be 22.03±3.952 Kg/m^2. The mean cholesterol level in the study group was found to be 212.87±23.23 mg/dl. The mean LDL level was 145.56±18.6 mg/dl. A statistically significant correlation was found between creatinine clearance and serum LDL (r=-0.491, p=0.006) and serum cholesterol levels (r=-0.464, p=0.01). The mean malondialdehyde levels in cases were found to be 6.34±0.44 nmol/ml in cases and 4.08+0.56 nmol/ml in controls. The mean IMT was found to be thicker in transplant patients (0.6285±0.07 mm) as compared to healthy controls (0.534±0.024 mm).

The mean hornocysteine levels were found to be 27.4±7.902 µmol/L in cases and 10.86±1.98 µmol/L in controls. The difference in means was found to be statistically significant (t=18.997, p<0.0001) (Table 1).

Table 1: Homocysteine levels in cases and controls.

Variable	N	Mean	Min	Max	Range	S.D.	P value
Cases	30	27.407	12.170	43.650	31.480	7.902	p<0.0001*
Controls	30	10.860	7.7	14.6	6.90	1.9816	

*Highly significant p value.

Table 2: Characteristics of transplant patients with and without cardiovascular events.

Variable	CVD+	CVD-	P value
N	6	24	-
Age (years)	44±13	30±7	0.049*
Sex			
Males	6	20	0.56
Females	0	4	
Donor			
Related	3	18	0.33
Unrelated	3	6	
Blood transfusion before transplant			
No	2	3	0.25
Yes	4	21	
Transplant duration (months)	50	35	0.545
Hemodialysis duration before transplant (months)	10	8	0.60
Albumin (gm/dL)	3.4±0.5	3.4±0.5	0.947
Systolic BP (mmHg)	156±11	146±16	0.122
Diastolic BP (mm Hg)	92±10	87±8	0.234
BMI (kg/m2)	20.56±2.44	22.40±4.21	0.183

*Significant p value.

Table 3: Characteristics of transplant patients with and without cardiovascular events

Variable	CVD+	CVD -	P value
Creatinine clearance (ml/min)	48.61±8.15	62.24±13.89	0.008*
Hemoglobin (gm/dl)	12.0±2.2	11.5±1.9	0.626
S. creatinine (mg/dl)	1.70±0.40	1.5±0.40	0.318
Proteinuria			
Present	2	5	0.60
Absent	4	19	
S. cholesterol (mg/dl)	216±19	212±24	0.68
LDL (mg/dl)	148.9±14.5	144.7±19.7	0.57
HDL (mg/dl)	32±7	33±12	0.78
MDA (nmol/ml)	6.48±0.31	6.30±0.47	0.30
Homocysteine (µmol/L)	29.4±4.2	26.9±8.6	0.33
LVH			
Present	4	6	0.14
Absent	2	18	
Mean IMT (mm)	0.681±0.03	0.615±0.071	0.001**

*Highly significant p value.

The mean Hcy (homocystiene) levels in the study group were higher in the males as compared to the females (29.51±6.141 µmol/L vs 13.76±1.69 µmol/L). Serum homocysteine levels were found to have a statistically significant correlation with serum creatinine (r=0.41, p=0.025), serum LDL (r=0.386, p=0.035) and serum malondialdehyde levels (r=0.822, p<0.0001).

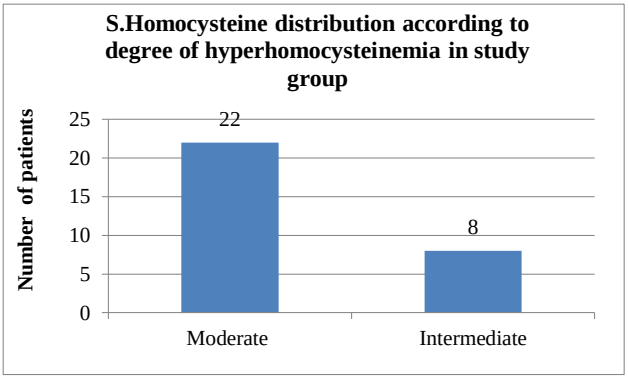

Figure 1: Degree of hyperhomocysteinemia in transplant patients.

Among the study group, 22 patients had moderate hyperhomocysteine and 8 patients had intermediate hyperhomocysteinemia (Figure 1).

There was no statistically significant relationship between homocysteine levels and transplant duration, mean IMT levels, proteinuria, and presence of left ventricular hypertrophy or choice of immunosuppressive regimen.

Occurrence of cardiovascular events

Of the 30 patients included in the study, 6 patients (20%) had evidence of cardiovascular event. Among these, 5 patients had history of angina that was subsequently confirmed with ECG and stress test. One patient had unstable angina that was diagnosed on the basis of history and changes on ECG. The troponin-T test was found to be negative. None of the patients had any symptoms or signs suggestive of TIA or stroke. There was no mortality among the study subjects during the period of study.

Plasma homocysteine failed to show as an independent risk factor for cardiovascular events. 20% of the renal transplant patients are at an increased risk of cardiovascular events. In the absence of other conventional factors (such as Diabetes, Smoking and Hyperlipidemia), age of the patient, creatinine clearance (index of graft function) and mean intima-media thickness are more closely related with cardiovascular events. New, emerging cardiovascular risk factors (e.g. Lipoprotein(a), high sensitivity C-reactive protein, fibrinogen, tissue plasminogen activator and plasminogen activator inhibitor-1) should be studied to design effective therapy to delay the progression of atherosclerosis and prolong the life of renal transplant recipients (Table 2 and 3).

DISCUSSION

In the present study, the mean fasting homocysteine level in transplant patients was found to be significantly higher as compared to controls. To the best of our knowledge, there are no published studies from India regarding homocysteine levels in renal transplant recipients. This result is in accordance with previous studies. Winkelmayer WC et al observed that elevated homocysteine levels were associated with 1.63 times increased risk of kidney allograft loss and are independently associated with CV events and mortality in stable RTRs.[7] Another prospective study observed that fasting homocysteine values were higher in those patients who experienced CV events than those who did not (31.5±10.3 vs 17.8±7.5; p<0.001) and correlated with both folate concentration (r =-0.3; p<0.01) and creatinine levels (r=0.54; p<0.001).[8]

Bostom observed in this study that hyperhomocysteinemia is linked to an almost 3 fold risk of de novo or recurrent CVD in ESRD and suggested as a risk factor for CVD in RTRs.[9] Two other studies could not find any association between hyperhomocysteinemia and patient or graft survival.[10,11]

Our study found a statistically significant correlation between tHcy and serum creatinine in transplant patients that is in accordance with earlier published reports. Massy et al in 1994 showed a relationship in the male patient group between serum tHcy (total homocystiene) and serum creatinine (r=+0.41, p=0.02).[12] Kumar et al also showed a positive correlation between tHcy and serum creatinine (r=+0.70, p=0.01) in their study of stable pediatric, adolescent and young renal transplant recipients.[13]

We observed that mean MDA levels were significantly higher in transplant patients. Further, a significant correlation was also found between tHcy and MDA levels in renal transplant patients. The serum LDL concentration also showed a positive and statistically significant correlation with tHcy levels (r=0.386, p=0.035, graph 19) in renal transplant patients. These findings may be a pointer towards a metabolic derangement indicating a higher oxidative stress in these patients.[14] High total cholesterol has been shown to increase the chance of having a MI in RTRs.[15] The carotid intima-media thickness is a strong predictor for cardiovascular disease.[16] In the study by Brzosko et al, IMT correlated significantly with age (r=0.5; p=0.001), time on dialysis prior to transplantation (r=0.6; p=0.001), fibrinogen (r=0.4; p=0.02), and t-PA (r=0.6; p=0.001).[17] According to the study by Toz et al in 102 randomly selected RT patients, a positive correlation was found between IMT and age, triglyceride level, and hematocrit.[18]

In the present study, the mean tHcy levels were found to be higher in the patients with proteinuria Proteinuria is an independent risk marker for both cardiovascular as well as renal disease The most plausible explanation is that urinary albumin leakage is the reflection of a generalized vascular dysfunction, particularly, the endothelial dysfunction.[19] Proteinuria has been reported in up to 30% of RTRs.[20] In renal transplantation, proteinuria at 1 year is coupled with a double-fold risk of CV death.[21] Persistent proteinuria can predict succeeding IHD and PVOD.[22] Low grade proteinuria discovered at early phase of post renal transplantation, is coupled with inferior graft and patient outcomes.[23] Both proteinuria and hypertension are linked with undesirable outcomes.[24]

In the present study, 20% of the patients had evidence of cardiovascular events during the six month follow-up. The mean homocysteine levels were higher in the group that developed cardiovascular events but was not found to be statistically significant. This may be due to the small sample size and short duration of follow-up. In the study conducted by Ducloux et al, 43 cardiovascular events were documented (coronary disease in 21, stroke in 10, and peripheral vascular disease in 12).[8] Plasma homocysteine concentrations were higher in those patients with cardiovascular disease than in patients without atherosclerotic complications. Two south Indian studies have also suggested that the elevated plasma homocysteine level found in patients of Indian origin is not independently associated with CAD.[25,26]

This study has several strengths. We studied role of homocysteine level as risk factor in the occurrence of cardiovascular events in renal transplant patients, an underexplored entity in India. It will add to existing literature. We included both traditional and non-traditional risk factors. Our study is unique for studying the northern Indian population. The study has some limitations as well, the most important being the small sample size. The duration of follow-up was only 6 months. The renal transplant patients should ideally be compared with patients having chronic kidney disease to better evaluate the cardiovascular risk factors. The obvious limitation of the study, however, is the lack of angiographic documentation in the study group for the presence of CAD. This could not be performed.

CONCLUSION

On the basis of empirical evidences of the current study it can be summarized that in patients with successful renal transplant, plasma homocysteine failed to show as an independent risk factor for cardiovascular events. In the absence of other conventional factors (such as diabetes, smoking and hyperlipidemia), age of the patient, creatinine clearance (index of graft function) and mean intima-media thickness are more closely related with cardiovascular events. New, emerging cardiovascular risk factors (e.g. lipoprotein, high sensitivity C-reactive protein, fibrinogen, tissue plasminogen activator and plasminogen activator inhibitor-1) should be studied to design effective therapy to delay the progression of atherosclerosis and prolong the life of renal transplant recipients.

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