A COMPARATIVE ANALYSIS OF HYPERHOMOCYSTEINEMIA IN HEMODIALYSIS PATIENTS VERSUS NORMAL CONTROLS

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ABSTRACT

Objective: *To compare the mean homocysteine level of ESRD patients undergoing regular hemodialysis with normal age and gender matched controls.*

Methodology: *This observational study was conducted at Dialysis Unit of Fauji Foundation Hospital, from January 2011 to October 2012. Cases included patients from 12-65 years age of either gender who were on regular hemodialysis for more than three months at a frequency of three times a week. Controls were chosen from the healthy relatives and hospital employees who had normal renal functions and were matched for age and gender. Total homocysteine in plasma was measured by HPLC and fluorescence detection.*

Results: *We included 55 hemodialysis patients and 55 controls. The mean age of cases and controls was 45.67±10.68 and 45.94±10.50 years respectively. There were a total of 24 (21.8%) males. The two groups were similar in respect to age and gender distribution. The mean homocysteine level of cases and controls was 17.69±5.17 and 12.85±3.27 µmol/L respectively (p =0.00).*

Conclusion: *Plasma homocysteine levels are significantly higher among homodialysis subjects as compared to normal controls.*

Key Words: *End Stage Renal Disease (ESRD), Chronic renal failure, Hemodialysis, Homocysteine levels.*

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INTRODUCTION

Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine. Elevated homocysteine levels occur in five to seven percent of the population[1]. Mean plasma homocysteine levels in Pakistani normal adults were found to vary from 10.8 +/- 4.1 µmol/L as reported by Aamir et al[2] to 17.95+/-8.4 µmol/l as reported by Iqbal et al[3]. Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular disease and for recurrent venous thromboembolism[4].

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The risk of premature and progressive cardiovascular disease is high in chronic uremic patients and accounts for more than 40% of the deaths in dialysis patients[5]. Prevention and treatment strategies demand precise knowledge of risk factors and of the possibility of modifying them with appropriate treatments. Hyperhomocysteinemia has been implicated as potential trigger of atherosclerotic complications in patients with chronic renal disease. End stage renal Disease (ESRD) patients exhibit elevated plasma homo-cysteine levels[6]. Experimental studies suggest that homocysteine may enhance lipoprotein oxidation, increases smooth muscle proliferation, induces endothelial dysfunction, in-duces endothelial activation of factor V, and reduces protein C activation in endothelial cells[7].

There is only one locally published study on homocysteine levels in ESRD population from Lahore[8]. This study was published a decade ago. Despite this and other international studies, checking of homocysteine levels and its lowering is not a part of routine management of ESRD patients in most dialysis centers in Pakistan. Therefore we think that more data on the subject will substantiate this evidence

so that recommendations can be formulated for routine homocysteine testing and lowering strategies in future for all ESRD patients.

METHODOLOGY

We conducted an observational study at Dialysis Unit of Fauji Foundation Hospital, Rawalpindi from January 2011 to October 2012 to compare the mean homocyteine levels in hemodialysis patients with normal controls. Hyperhomocysteinemia was defined as plasma homocysteine levels > 15 µmol/L. The calculated appropriate sample size using the WHO calculator was 55 hemodialysis patients and 55 controls. Patients and the controls were included in the study after taking an informed consent. These patients were recruited from the dialysis unit through non-probability convenient sampling. Cases included patients from 12-65 years age of either gender who were on regular hemodialysis for more than three months at a frequency of three times a week. Controls were chosen from the healthy relatives and hospital employees who had normal renal functions. Controls were matched for age and gender to minimize bias. Patients with hepatic dysfunction, pregnancy, hypothyroidism, acute or chronic infection, cancer, current or recent (within 6 months) use of folic acid or B12 supplements were excluded based on history. The renal functions of the controls were checked and they were included if their urea and creatinine levels were in the normal range. For homocysteine analyses, fasting blood was collected in EDTA-containing tubes; the plasma was separated within 15-20 min. and stored at -20°C. Total homocysteine in plasma was measured by HPLC (High Performance Liquid Chromatography) and fluorescence detection. Obtained data was converted into variables which were analyzed using Statistical Package for Social Sciences (SPSS) version 12. Independent sample t–test was used to compare homocysteine levels in both groups. A P value less than 0.05 was taken as significant.

RESULTS

We included 55 hemodialysis patients and 55 controls. The patients and controls were matched for age and gender. The age ranged from 14 to 65 years with a mean of 45.8±10.55 years. The mean age of the cases and controls was 45.67±10.68 and 45.94±10.50 years respectively; this difference was not statistically significant; p =0.893. There were a total of 24 (21.8%) males out of which 12 were among cases and 12 age matched males were chosen in the controls. Hence the two groups were similar in these demographic characteristics.

The homocysteine level of all subjects (both cases and controls) ranged from 7 to 38 µmol/L with a mean of 15.27±4.95 µmol/L. The mean homocysteine level of cases and controls was 17.69±5.17 and 12.85±3.27 µmol/L respectively; this difference was statistically significant; p =0.00 [Figure 1]. Among the cases 38 (69.1%) had hyperhomocysteinemia and among the controls 9 (16.4%) had hyperhomocysteinemia; hence the proportion of hyperhomocysteinemia was significantly more among the hemodialysis population; p= 0.00 [Figure 2]. The OR of hyperhomocysteinemia among the hemodialysis group as compared to controls was 11.42 (95% CI= 4.57-28.53).

Figure 1: Homocysteine levels of hemodialysis group and controls

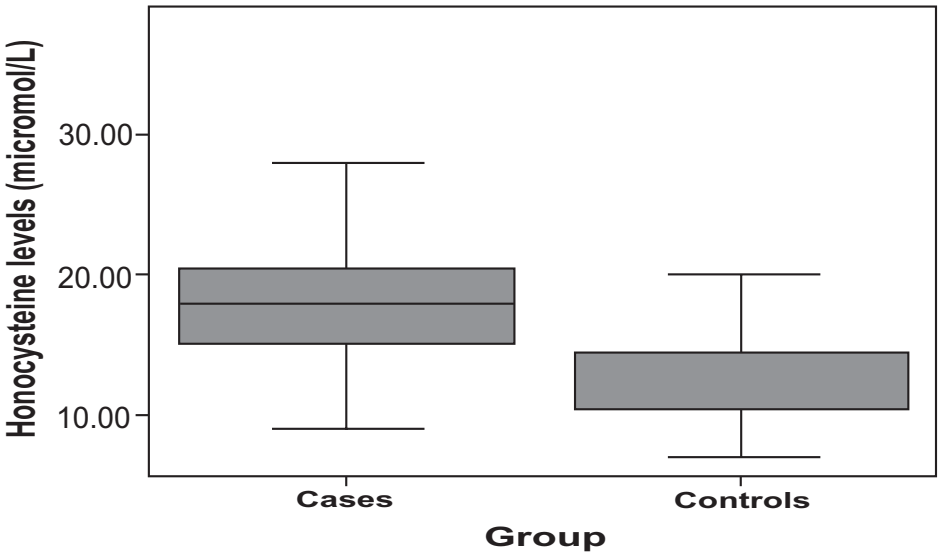
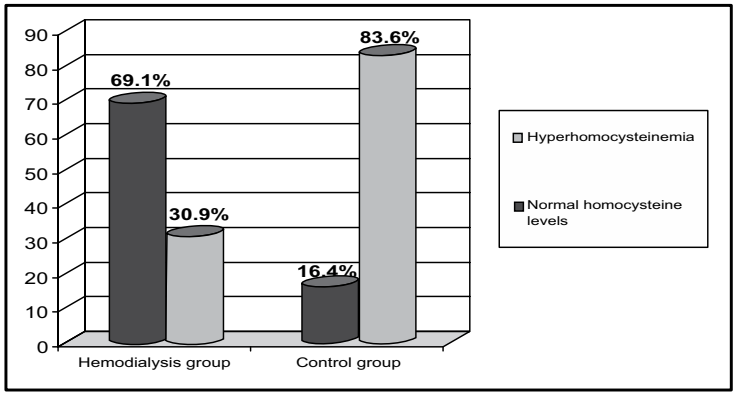


Figure 2: Hyperhomocyteinemia among hemodialysis group and controls



DISCUSSION

In our study we have found that the plasma homocysteine levels were significantly higher among homodialysis subjects as compared to controls. We found that the fasting homocysteine level of hemodialysis subjects was 17.69±5.17 umol/L and that of age and gender matched controls was 12.85±3.27 µmol/L. The homocysteine level of normal controls in studies of Salahuddin et al[9], Aamir et al[2] and Iqbal et al[10] was 10.57±0.31, 10.8±4.1 and 16.4±4.9 u mol/I, respectively. The mean homocyteine levels of hemodialysis patients and normal controls as reported by Shakeri et al[11] was 46.22±6.2 and 10.21±5.23 respectively; ($p<0.001$). Similar results were reported in ESRD patients as compared to controls by Choudry et al from Shaikh Zayed Hospital Lahore[12].

Most studies so far have indicated normal plasma homocysteine level to be in the range of 5 to 15 umol/I[13]. Using the cut-off value of > 15 µmol/L to define hyperhomocysteinemia we found the frequency of hyperhomocysteinemia among hemodialysis versus control groups to be 69.1% and 16.4% respectively. Robinson et al[14] showed that there was a prevalence of high homocysteine level in the dialysis patients and they also showed that this same dialysis treated population had a significant deficiency of vitamin B 12 and B 6 levels.

In a study by Berneih et al[15] homocysteine and folate levels were measured in 83 stable HD patients, 99% had abnormal homocysteine with a mean level of 27±10.8 mmol/L. In another study by Lovcic et al[16] an increased concentration of total homocysteine (mean 24.76 + 11.04 micromol/L) was observed in 85% of hemodialysis subjects. Silva de Almeida reported a prevalence of hyperhomocysteinemia (>15 µmol/L) among hemodialysis patients to be 85.7%[17].

High prevalence of deficiency of folate and vitamin B6 appears to be the major cause of hyperhomocysteinemia in hemodialysis population. Different therapies including folate supplementation and N-acetylcysteine therapy have been tested to reduce homocysteine level in dialysis subjects[18]. The deficiency of these vitamins could be due to anorexia, vomiting and associated nutritional deficiencies among hemodialysis subjects. In Pakistani population the association of CAD has been linked to hyperhomocysteinemia by Salahuddin et al[9] and Aamir et al[2]. In these studies no underlying factor contributing to the hyperhomocysteinemia has been identified however in other studies by Iqbal et al[10] and Akhtar et al[19], hyperhomocysteinemia in both the CAD as well as the control population has been ascribed to dietary deficiencies of folate and vitamin B6. In another study by Fellah et al[20] mean homocysteine concentration was 2 to 3 times higher in ESRD patients than in subjects with normal renal function, however in our study the hemodialysis population had 1.4 fold higher homocysteine levels as compared to controls.

Among other determinants of homocyteine levels, renal function is a major factor and there is an inverse relationship between the glomerular filtration rate and plasma homocysteine level throughout the whole range of renal function[21]. Homocysteine has been associated with atherosclerosis and coronary artery disease[22]. Wald et al[23] found that for every 5-µmol/L increase in homocysteine, the risk of ischemic heart disease increased 20% to 30%. Heinz et al found that a similar increase in homocyteine was associated with a 9% increased risk of CVD among patients with ESRD[24]. Among patients treated with hemodialysis or peritoneal dialysis, the prevalence of CAD is approximately 40% and the prevalence of left ventricular hypertrophy is approximately 75%. CVD mortality in dialysis patients is 10 to 20 times higher than in the general population[25]. Hyperhomocysteinemia in the ESRD population has been con-

sistently shown to be responsible for the increased incidence of coronary events in this population[26].

Strategies aimed at reducing the raised homocysteine level may result in a reduced incidence of coronary events in these patients.

CONCLUSION

Plasma homocysteine levels are significantly higher among homodialysis subjects as compared to normal controls. Strategies aimed at reducing the raised homocysteine level may result in a reduced incidence of coronary events in these patients. Future studies can be planned to aim at finding therapies to reduce this risk factor in dialysis patients.

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CONTRIBUTORS

UB conceived the idea and worked for the acquisition of the data. KN designed the study protocol and wrote the complete manuscript. Both the authors contributed significantly to the research that resulted in the submitted manuscript.