

## Research Article

# Plasma homocysteine and total antioxidant status in diabetic chronic kidney disease and diabetic renal allograft recipients: effect of folic acid therapy

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## ABSTRACT

**Background:** Indian population is usually deficient in folic acid. Aim was to study the plasma homocysteine and antioxidant status in type 2 diabetics and healthy individuals before and after folate therapy.

**Methods:** This study was done in four groups of 25 cases each. These included: type 2 diabetics with end stage diabetic chronic kidney disease (CKD) (group A); diabetic renal allograft recipients with normal and stable graft function (group B); uncomplicated type 2 diabetics (group C); and age and sex matched healthy controls (group D). The serum homocysteine and total antioxidant status (TAS) were measured at baseline and after 4 weeks of folate therapy.

**Results:** The plasma homocysteine levels were 18.163.80, 16.150.66, 12.480.82 and 23.361.61 mol/L in group A, B, C and D respectively. The homocysteine levels were significantly low in all diabetic groups when compared to healthy controls. The plasma homocysteine were significantly elevated in stage 5 diabetic CKD and diabetic renal transplant recipients as compared to uncomplicated diabetics. After four weeks of folate therapy, there was a significant decrease of homocysteine in all the groups. The mean values of TAS were 1.42 0.18, 1.49 0.18, 1.17 0.06 and 1.60 0.86 pg/ml in group A, B, C and D respectively. There was no significant correlation between diabetic groups and healthy controls. No change was observed in TAS levels after folate therapy.

**Conclusions:** Our results show significant hyperhomocysteinemia in healthy Indians. Plasma homocysteine were significantly low in all diabetic groups as compared to healthy individuals. We suggest supplementation of Indian diet with folic acid.

**Keywords:** Diabetic chronic kidney disease, Renal allograft recipients, Type 2 diabetes mellitus

## INTRODUCTION

Homocysteine, a sulfur-containing amino acid, is an intermediate metabolite of methionine metabolism. Homocysteine once formed from methionine is converted back to methionine with the help of folate. High levels of

homocysteine are believed to promote the formation of oxidation products that can damage the endothelial cells by excessive sulfation of collagen.<sup>1</sup> In animal experiments, plasma homocysteine levels are reduced in streptozotocin-induced diabetic rats and rise with institution of insulin treatment.<sup>2</sup> Patients of type 2

diabetes mellitus who have preserved  $\beta$ -cell function, and who are insulin resistant and hyperinsulinic are likely to have higher concentrations of plasma homocysteine.<sup>3</sup> These same patients when they lose beta-cell reserve might then show a decline in homocysteine concentrations. The kidneys have an important role in homocysteine metabolism; as renal function declines, homocysteine concentrations increase.<sup>4</sup>

The aim of this study was to assess the plasma homocysteine and total antioxidant status in patients with diabetic stage 5 chronic kidney disease (CKD) and the diabetic renal allograft recipients, before and after one month of oral folate therapy, and to compare the results with the diabetics without nephropathy and the healthy controls.

## METHODS

This prospective study was conducted in a tertiary care medical college hospital in North India. The Institutional Ethical Committee on human research and the State Medical University approved the study. The study included patients of type 2 diabetes mellitus. These included: 25 cases of type 2 diabetes mellitus with diabetic stage 5 chronic kidney disease on intermittent hemodialysis (group A); 25 cases of type 2 diabetes mellitus who had received renal allograft at least one year back and had stable and normal graft function (group B); 25 cases of diabetes mellitus without complications (group C); and 25 age and sex matched healthy individuals (group D). Estimation of glomerular filtration rate (e-GFR) was made from serum creatinine using CKD-EPI equation.  $GFR \leq 15$  ml/minute or need of dialysis was considered as stage 5 CKD. The diagnosis of uncomplicated diabetes mellitus was based on normal fundus examination and absence of Microalbuminuria and neuropathy. Patients on metformin and fibric acid derivatives were not included in the study as these can raise plasma homocysteine levels.<sup>5</sup> Since Indian diet is not fortified with folic acid, plasma homocysteine level  $\geq 15\mu\text{mol/L}$  was considered as hyperhomocysteinemia.

After taking an informed written consent, a detailed history and physical examination including fundoscopy was carried out in each case. Various base line investigations were carried out including hemogram, routine urine examination, 24 hour urinary proteins (Microalbuminuria in group C and D), blood sugar, serum HbA1c, blood urea, serum creatinine, serum electrolytes, serum calcium, serum phosphorus, electrocardiogram, chest X-ray and renal ultrasound. Nerve conduction study was done in patients of group C. Fasting plasma homocysteine was estimated using Micro plate Enzyme Immunoassay and the serum total antioxidant status (TAS) using kit from Randox Diagnostics. All subjects received 5 mg a day of tablet folic acid (5 mg thrice daily in stage 5 CKD) and the plasma homocysteine and TAS levels were repeated after four weeks of folate therapy. The results were expressed

as mean $\pm$ SD and compared using student's t-test.  $p < 0.05$  was considered significant.

## RESULTS

There were 72 males and 28 females with mean age of  $47.8 \pm 11.91$  and age range of 15 to 75 years. As depicted in table 1, the mean values of plasma homocysteine were significantly low in all diabetic patient groups when compared to healthy controls. Amongst the various diabetic patient groups, the mean values of plasma homocysteine were significantly higher in end stage diabetic CKD when compared to diabetics without nephropathy but insignificantly higher as compared to stable diabetic renal allograft recipients. Mean plasma homocysteine were also significantly higher in normally functioning diabetic renal allograft recipients as compared to uncomplicated diabetes mellitus patients. After a four week folate therapy, there was a significant decline in mean values of plasma homocysteine in all the groups. The prevalence of hyperhomocysteinemia ( $\geq 15\mu\text{mol/L}$ ) was 52%, 52%, 24% and 92% in group A, B, C and D respectively that decreased to 8%, 24%, 16% and 44% respectively after 4 weeks folate therapy.

The difference in the mean values of serum TAS amongst the patient groups and the healthy controls as well as after folate therapy was insignificant.

## DISCUSSION

One of the striking observations in our study was that the mean values of plasma homocysteine were significantly elevated in healthy controls as compared to all three diabetic patient groups. Hyperhomocysteinemia was present in 94% of healthy controls. The increased levels of homocysteine in our healthy population may be due to folic acid and/or vitamin B12 deficiency. There could be ethnic related reasons as similar findings have been observed by others.<sup>6,7,8</sup> Although, the serum folate and vitamin B12 were not measured in the present study, a four week folate therapy showed a significant decrease in serum homocysteine levels in control subjects, suggesting thereby a possible folate deficiency in the diet of Indian population. Although, the mechanism responsible for the reduction of homocysteine levels in diabetics is unknown, it has been suggested that the demethylation pathway may be disturbed in these patients.<sup>9</sup>

Amongst the diabetic groups, the mean values of plasma homocysteine were significantly higher in diabetic end stage renal disease and in stable diabetic renal allograft recipients as compared to uncomplicated diabetes mellitus. An elevated homocysteine concentration in type 2 diabetes suggests an association between hyperhomocysteinemia and deterioration of renal function. Kidney function is critical for homocysteine clearance, and hyperhomocysteinemia occurs frequently in patients with renal failure.<sup>10,11,12</sup> The observation of hyperhomocysteinemia in about half of our diabetic renal

transplant recipients has also been reported by others.<sup>13,14</sup> Folic acid has been shown to lower homocysteine levels in several studies.<sup>8,15,16</sup>

**Table 1: Patient characteristics.**

Variables (Mean±SD)	Group A (n=25)	Group B (n=25)	Group C (n=25)	Group D (n=25)
<b>Serum creatinine (mg/dl)</b>	8.56 ±2.44	1.32 ±0.52	1.0 ±0.34	0.79 ±0.24
<b>e-GFR (CKD-EPI) (ml/mt)</b>	8.16 ±3.80	63.68 ±4.03	92.32 ±9.69	111.53±8.22
<b>Plasma homocysteine (µmol/L)</b>				
Base line	18.16±3.80* †	16.15 ±0.66* †	12.48 ±0.48 *	23.36 ±1.61
After folate therapy	10.47±1.74 ‡	13.14 ±0.57 ‡	9.83 ±0.77 ‡	17.53 ±1.61 ‡
<b>Total antioxidant status (pg/ml)</b>				
Base line	1.42 ±0.18	1.49 ±0.18	1.17 ±0.06	1.60 ±0.86
After folate therapy	1.24 ±0.18	1.17 ±0.11	1.06 ±0.06	1.09 ±0.08

\*p<0.05 when compared with healthy controls (group D), † p<0.05 when compared to uncomplicated diabetics (group C), ‡ p<0.05 when compared the base line values

Oxidative damage by free radicals has been implicated in the pathogenesis of vascular disease in diabetes. There are many endogenous factors (antioxidants, vitamins, antioxidant enzymes, metal ion chelators) that can serve as endogenous modulators of the production and action of ROS. Clinical trials that investigated the effect of antioxidant vitamins on the progression of diabetic complications gave negative or inconclusive results.<sup>17, 18</sup> We did not observe any significant difference in the values of total antioxidant status in diabetics with end stage kidney disease, uncomplicated diabetes mellitus, stable renal allograft recipients and healthy individuals.

In conclusion, the results of our prospective study show a significant hyperhomocysteinemia in most (92%) of healthy controls with a significant lowering after folate therapy suggesting dietary folate deficiency in our healthy population. Also there was significant hyperhomocysteinemia in approximately half of diabetic end stage renal disease and diabetic renal allograft recipients and 1/4th of type 2 diabetics without any complications.

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