

## Effect of Glycemic Control on Homocysteine Levels in Type 2 Diabetic Patients without Cardiovascular Disease

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Article information	Abstract
<p>Article history: Received: 4 Nov 2012 Accepted: 20 Dec 2012 Available online: 11 May 2013 ZJRMS 2014; 16(1): 23-27</p> <p>Keywords: Glycemic control Homocysteine HbA1c Diabetes</p> <p>*Corresponding author at: Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran. E-mail: hafezheydari@yahoo.com</p>	<p><b>Background:</b> An increased serum homocysteine level was accepted as an important risk factor for vascular disease, including coronary atherosclerosis. However, there was no data about the importance of glycemic control on homocysteine levels in type 2 diabetic patients without CVD. The aim of this study was to investigate association between serum homocysteine concentrations and glycemic control in type 2 diabetic patients without CVD.</p> <p><b>Materials and Methods:</b> Out of 100 diabetic patients, 50 were good glycemic control and 50 patients were poor glycemic control. Also we tested fifty healthy volunteers as controls. Degree of glycemic control in diabetic patients was evaluated by HbA1c concentration measurements. Serum homocysteine level was measured in patients with good or poor glycemic control, and healthy controls. The correlation of HbA1c and homocysteine concentrations was investigated.</p> <p><b>Results:</b> The results indicated HbA1c concentration and total serum levels of homocysteine in patients as whole are significantly higher than healthy subjects. HbA1c concentration is significantly higher in subgroup with poor glycemic control compared to subgroup with good glycemic control and healthy control group. However, there is no significant difference in homocysteine serum levels of patients with good and poor glycemic control.</p> <p><b>Conclusion:</b> The findings suggest elevation of serum homocysteine level in patients with type 2 diabetes, however there is not significant correlation between homocysteine concentrations and glycemic control in type 2 diabetes patients.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

Diabetes mellitus (DM) is a group disorder characterized by hyperglycemia resulting from defects in insulin secretion or insulin action [1]. Chronic hyperglycemia in diabetes may cause permanent damage to the eyes, nerves and kidneys [2]. Prevalence of diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity [3].

Cardiovascular disease (CVD), being 2-4 folds more prevalent in type 2 diabetes mellitus, is still keeping its importance as the leading cause of death in this patient group [4]. Thus, the modulation of cardiovascular risk factors in these patients is very important. Homocysteine is an accepted risk factor for CVD. Hyper homocysteinemia is known to be associated with atherosclerosis, and this association is stronger in individuals with type 2 diabetes than in non-diabetic subjects [5].

Homocysteine is a thiol containing amino acids that produced in methionine metabolism as intermediate [6]. This non-protein amino acid included in cardiovascular disease pathogenesis in several ways such as enhancement of blood coagulation [6], oxidative stress [7], endothelium dysfunction [8] and cardiomyocyte

dysfunction [9]. Studies have been shown that serum homocysteine levels are elevated in diabetic as well as non-diabetic patients with cardiovascular disease [5].

Current guidelines for treating patients with type 2 diabetes mellitus are based on glycemic control. Glycemic control is mandatory for prevention of diabetes complications [10].

Microvascular complications, including nephropathy, retinopathy, and neuropathy, are strongly related to glycemic control [11]. However, vascular complications may progress in patients with good glycemic control and may appear even in undiagnosed patients [12]. So far numerous studies investigate homocysteine as cardiovascular risk factors in diabetes patients; however, study results about the relationship of homocysteine levels with glycemic control are contradictory and require further investigation.

The aim of this study was to investigate relation of glycemic control with serum levels of homocysteine. For this purpose serum homocysteine level in type 2 diabetes mellitus patients with good and poor glycemic control have been compared with age and sex matched healthy controls.

## Materials and Methods

### Study participants

The research protocol was approved by ethical committee of medical science university of Mashhad. Included patients selected out from type 2 diabetes patients referred to endocrinology of Ghaem clinics of Mashhad city. In these patients diabetes were diagnosed with world health organization definition: having fasting serum glucose  $\geq 126$  mg/dl. For all patients questionnaires completed. Medical history, medications, smoking, alcohol consumption, folic acid, B<sub>12</sub> and B<sub>6</sub> consumption, thyroids diseases were recorded.

HbA1c concentration measured in whole blood by affinity chromatography method. HbA1c concentration  $<7\%$  and  $>8\%$  considered as good and poor control cut off respectively [13]. Patients were categorized to two subgroups: good and poor glycemic controls. Finally fifty patients with good glycemic control and fifty patients with poor glycemic control were selected out and included to study. As control, fifty age and sex matched healthy volunteers were studied. Healthy volunteers have normal fasting glucose (70-110 mg/dl) and HbA1c concentration (4.3-5.9%).

### Blood collection

Peripheral blood samples were collected in tubes containing EDTA as anticoagulant and without anticoagulant at 8.00 am after an overnight fasting. HbA1c concentrations were determined in whole blood immediately. Serum separated by centrifuging at 3000 r.p.m for 10 minutes and stored in  $-20$  until analyzed. Serum total homocysteine level was measured for diabetic patients and healthy subjects.

### Homocysteine (Hcy) assay

The serum homocysteine level was analyzed by enzymatic immunoassay method (EIA) by Axis-Shield Homocysteine Enzyme Immunoassay (EIA) kits. Quantification limit of this assay (CV $<20\%$ ) is 1.0  $\mu\text{mol/L}$ . Briefly, in this method, protein-bound Hcy is reduced to free Hcy and then reacts with serine catalyzed by cystathionine beta synthase to form L-cystathionine. Cystathionine is cleaved to homocysteine, pyruvate and ammonia by cystathionine beta lyase. Pyruvate is converted to lactate with NADH as coenzyme by lactate dehydrogenase. Concentration of homocysteine is directly proportional to the rate of NADH conversion to NAD.

### Data analysis

Data were analyzed using a SPSS-14 program and expressed as mean $\pm$ SD continuous variables with normal distribution. The student *t*-test was used to test the significance of differences between the two means. The associations of serum Hcy levels with glycemic control index were assessed by Pearson's and Spearman's correlations. Differences were considered significant at  $p<0.05$ .

## Results

The three groups were well-matched for number (good glycemic control: 50 patients, poor glycemic control: 50 patients and healthy control: 50 volunteers), sex (good glycemic control: 22 male and 28 female, poor glycemic control: 20 male and 30 female and healthy control: 25 male and 25 female) and age (good glycemic control:  $42.48\pm 11$ , poor glycemic control:  $49.33\pm 9$  and healthy control:  $46\pm 4$ ).

Total HbA1c concentration in diabetes patients is significantly higher than healthy controls. Also HbA1c concentration in poor glycemic control is higher than good glycemic control ( $9.1\pm 1$  vs.  $6.7\pm 0.45$ ). The results of total HbA1c in the subgroups with good and poor glycemic control are compared in figure 1. Homocysteine levels were significantly higher in diabetic patients compared to controls (mean  $27.1\pm 2.23$  vs.  $15.45\pm 5.42$   $\mu\text{mol/L}$ ,  $p<0.001$ ) figure 2. The results of serum levels of homocysteine in the subgroups with good and poor glycemic control are compared in figure 3. Serum level of homocysteine weren't statistically significant difference in these subgroups. Both subgroups included the same of hyperhomocysteinemia (means  $27.82\pm 4.11$  vs.  $26.45\pm 3.04$   $\mu\text{mol/L}$  in patients with good and poor glycemic control respectively). Pearson's correlation coefficients among Hcy and HbA1c were ( $r=0.14$ ).

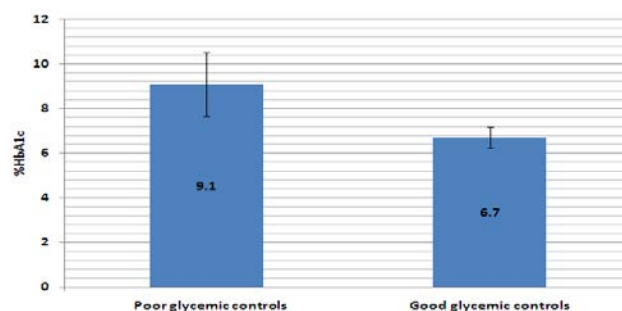


Figure 1. Comparison of total HbA1c in good and poor glycemic control subgroups

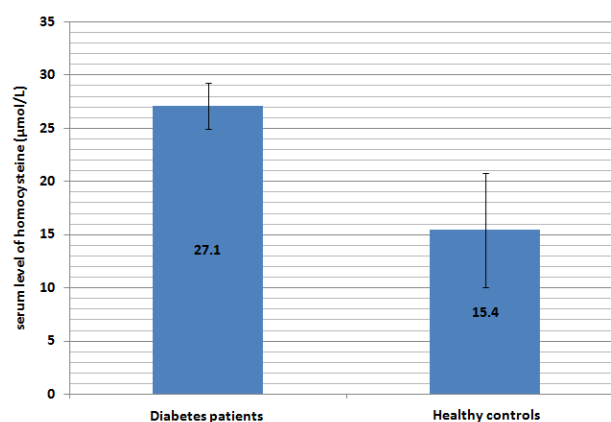
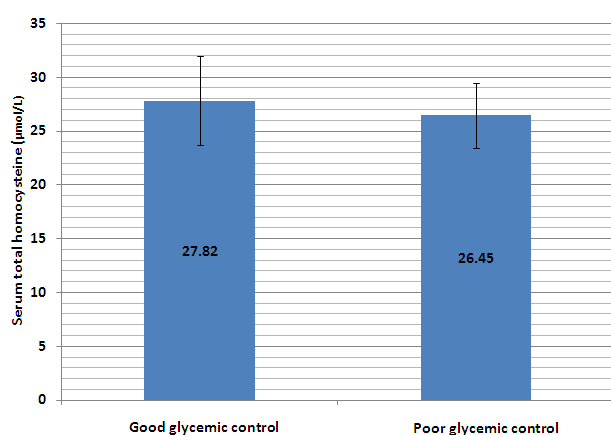


Figure 2. Comparison of serum total homocysteine in diabetes patients and healthy controls



**Figure 3.** Comparison of serum total homocysteine in good and poor glycemic control subgroups

## Discussion

In recent years, numerous studies have proposed mild hyperhomocysteinemia play a role in development of cardiovascular disease [14]. However relation of glycemic control and homocysteine level as a cardiovascular risk factor is not investigated properly and results of various studies are inconsistency. Therefore, this study was designed and conducted to assess relation of glycemic control and homocysteine level.

Our results show HbA1c concentration in diabetes patients as whole and in good and poor glycemic control subgroups considered separately were significantly higher than healthy volunteers. On base of previous studies, HbA1c concentration is index of glucose concentration and high levels of HbA1c indicate higher levels of glucose [15].

In agreement with previous studies [16], the results of the present study show serum level of homocysteine are significantly higher in diabetic patients as a whole than in the controls. All of diabetes patients were studied in this study has mild hyperhomocysteinemia. Mild hyperhomocysteinemia defined as medical condition that characterized by serum total homocysteine in the range of 15-30 µmol/l [17]. These results confirm presence of hyperhomocysteinemia in diabetic patient, as reported by some of previous studies [5, 18, 19]. Type 2 diabetes patients are prone to hyperhomocysteinemia for several reasons. Some authors suggest relationship between homocysteine level and insulin resistance [20]. Also long term therapy by metformin lead to lowering folic acid and B12 and finally accumulation of homocysteine in body's fluids [21]. Folic acid and B<sub>12</sub> are coenzymes of homocysteine metabolic pathways.

Interestingly, some of healthy subject that studied in this study have mild hyperhomocysteinemia. Mild hyperhomocysteinemia is common in general population of some country. Some of previous studies report high prevalence of mild hyperhomocysteinemia in our population. Fakhrzadeh et al. report prevalence of mild hyperhomocysteinemia in Iranian population is 47.6% [22]. In other study Bandarian et al. report prevalence of

mild hyperhomocysteinemia in urban population of Iran 52% [22]. Folic acid deficiency [23], B<sub>12</sub> deficiency [23], genetic factors [24] are probable reasons of mild hyperhomocysteinemia in healthy subjects. In our view, reasons of mild hyperhomocysteinemia in our healthy population need further investigation.

In our study, although homocysteine level in diabetic patients is higher than controls but there is no significant differentiate between two subgroups. This finding indicates good glycemic control and lowering blood glucose don't affect serum homocysteine and there isn't an important relationship between homocysteine and glycemic control.

The result of various studies about impact of glycemic control on plasma homocysteine is inconsistent. Some of previous studies suggested there is no significant correlation between serum homocysteine and glycemic control. Hoogeven et al. report there is no significant association between serum homocysteine and HbA1c concentration [25]. In another study Aghamohammadi et al. investigate association of plasma level homocysteine with good and poor glycemic control in 70 men with type 2 diabetes. Aghamohammadi et al. suggested there is no statistically significant correlation between homocysteine and glycemic control [26].

Pouwels et al. investigates effect of improved insulin sensitivity and glycemic control on plasma homocysteine. They confirmed glycemic control don't influence plasma homocysteine level [27, 28]. Our findings are consistent with result of these studies. In other hand, there are some studies that glycemic control of diabetes may influence Hcy levels [19, 29, 30]. In these studies proposed serum homocysteine level might be as one of the factors underlying the link between hyperglycemia and cardiovascular risk in diabetic patients [19]. These authors have shown that patients with poor glycemic control of diabetes had significantly higher Hcy levels in comparison to diabetics with normal HbA1c levels. Statistically significant correlation between Hcy concentration and HbA1c was also noticed in other studies.

El-Sammak et al. evaluate association of glycemic control, age and C677T mutation of the methylenetetrahydrofolate reductase gene with plasma level homocysteine in selected Egyptian subjects. They report that there is a positive correlation between the plasma Hcy and impairment of both renal functions and glycemic control [31]. Passaro et al. investigate relationship of homocysteine levels and degree of metabolic control in type 2 diabetic patients. In this study patients followed up for three years. Result of this study showed there is significant positive correlation between metabolic control and homocysteine levels [32].

Although our result indicated that glycemic control has no effect on homocysteine levels, in our view the relationship between homocysteine levels and glycemic control is not yet clear and need further investigation. Finally our study has several strengths and limitations compared with prior studies. Strengths of our study include 1) an adequate sample size for each study group

2) fasting specimens used 3) an accurate and precise method for total homocysteine and HbA1c determination used 4) standard protocol for diagnosis of DM and classification of glycemic control used. Our study has several limitations. We were not assessed some of factors linked to homocysteine such as red cell folate levels [33], genetic factors [18], depression [34] and life style habits [35]. In conclusion, result of this study and evidence of some previous studies suggest good glycemic control isn't sufficient for serum homocysteine reduction and other functions such folic acid or B<sub>12</sub> fortification are essential.

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

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