

The role of Glycemic Control on Homocysteine Levels in Type 2 Diabetic Patients in Iraqi people

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Abstract

Hyperhomocysteinaemia has emerged as a novel risk factor for cardiovascular disease . In non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease (CVD) is one of the major macrovascular complication . The present study was conducted to examine the effect of homocysteine concentration and glycemic control in type 2 diabetic patients . Sixty type 2 diabetic patient were studied .These patients were categorized to good glycemic control (30) , poor glycemic control (30) diabetic patients and (30) healthy volunteers were enrolled in to the study as controls . The degree of glycemic control in diabetic patients was evaluated by HbA_{1c} concentration.

Significant increases were obtained in HbA_{1c} concentration and total serum of homocysteine levels in patients as whole compared with corresponding control values .Although HbA_{1c} concentration was significantly higher in good glycemic control compared to poor glycemic control but there was no significant difference in homocysteine levels in patients with good and poor glycemic control .

These results suggest that there is an elevation of serum homocysteine level in type 2 diabetic patients , however there is no association between homocysteine levels and glycemic control in patients with type 2 diabetes .

Keywords : typ2 diabetes ,homocysteine , glycemic control, HbA_{1c}

الخلاصة

يعتبر ارتفاع مستوى الحامض الاميني الهوموسيستين في الدم كعامل خطر لأمراض القلب والاعوية الدموية على المدى الطويل .تعتبر الامراض القلبية الوعائية هي واحدة من مضاعفات الاعوية الكبيرة في المرضى المصابين بداء السكري غير المعتمد على الانسولين .تهدف الدراسة الحالية لمعرفة تأثير تركيز الهوموسيستين والسيطرة على مستوى سكر الدم في المصابين بداء السكري النوع الثاني . تم دراسة ٦٠ مريض من مرضى السكري النوع الثاني حيث صنفوا الى ٣٠ مريض ذو سيطرة جيدة على سكر الدم و ٣٠ مريض ذو سيطرة ضعيفة ومقارنتهم مع ٣٠ متطوع من الاصحاء . تم استخدام معيار HbA_{1c} لتقييم السيطرة على السكري في المرضى المصابين بالسكري .حصلت زيادة معنوية في تركيز HbA_{1c} ومستوى التركيز الكلي للهوموسيستين للمصابين ككل ومقارنتها مع القيم الطبيعية .تبين كذلك زيادة معنوية في تركيز HbA_{1c} في المرضى ذوي السيطرة الجيدة مقارنة مع ذوي السيطرة الضعيفة . بينما لا توجد اختلافات معنوية في مستويات الهوموسيستين في المرضى فيما بينهم .نستنتج من هذه الدراسة حصول ارتفاع في مستويات الهوموسيستين لمرضى داء السكري النوع الثاني مع عدم وجود ارتباط بين مستويات الهوموسيستين والسيطرة على داء السكري النوع الثاني .

Introduction

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control (ADA.2015). Diabetes, mainly Non insulin dependent diabetes mellitus (NIDDM), affects nearly 150 million people worldwide and 30% rise is predicted by 2025 due to increased rate of obesity and the ageing population living in industrial countries (Chandrakant *et al.*,2015). Due to the excessive cardiovascular mortality and morbidity associated with diabetes, it can be redefined as a state of premature cardiovascular death, associated with chronic hyperglycemia and sometimes with blindness and renal failure(Newsholme *et al.*,2007).

Homocysteine, a sulfhydryl-containing amino acid, is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine (Ganguly and Alam ,2015). The primary source of methionine is animal protein (Hankey and Eikelboom ,1999). The normal range of homocysteine is 5 to 15 $\mu\text{mol/L}$. Elevated serum homocysteine beyond the normal range ($>15 \mu\text{mol/L}$) is traditionally referred to hyperhomocysteinemia. Hyperhomocysteinemia is further subcategorized into moderate (15-30 $\mu\text{mol/L}$),intermediate (30-100 $\mu\text{mol/L}$), and severe ($>100 \mu\text{mol/L}$) (Hammad,2013).

Increments in Elevated plasma Hcy has been recognized as an independent risk factor for cardiovascular disease (CVD)as well as increased morbidity and mortality associated with CVD(Hwang *et al.*,2013). Similarly, diabetes mellitus is a known risk factor for atherosclerosis, peripheral vascular disease,and CVD . Moreover, there is evidence that high levels of tHcy contribute to the acceleration of CVD in diabetics . Levels of tHcy are sometimes,but not always, higher in diabetics(Robbins *et al.*,2005).

Accordingly,diabetes treatment guidelines emphasize on that improving good glycemic control reduces risk of microvascular complications and cardiovascular disease(Inzucchi *et al.* ,2012).Microvascular complications, including nephropathy, retinopathy, and neuropathy, are strongly related to glycemic control (Skyler *et al.*,2009).

However, vascular complications may progress in patients with good glycemic control and may appear even in undiagnosed patients (Del Prato *et al.*,2005).

In order to clarify the issue of the real link between serum homocysteine levels and glycemic control a prospective study in two groups of type

2DM patients with good and poor glycemic control which carried out and compared with controls.

Material and Methods

The study was conducted during the period from March 2015 to December 2015 in Merjan hospital in Hilla city , Babylon province, Iraq. The study group included patients with type 2 diabetes .These patient were diagnosed with world health organization definition :having fasting serum glucose \geq 126 mg/L or 7.0 mmol/L .Physical characteristics like height, weight were recorded to calculate BMI by dividing weight in kg by height in meter square .And all questionnaires were completed, including a medical history , medications ,smoking , alcohol consumption ,folic acid B12 and B6 consumption , thyroids diseases (Chandrakant *et al.*,2015).

Patients were categorized in to two subgroups :good and poor glycemic controls. Thirty patients with good glycemic control(HbA1c<7%) ,thirty patients with poor glycemic control(HbA1c>8%) were selected out and included to study .Finally results were compared between healthy volunteers and patients.

Blood Sampling :-

5ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 AM- 9AM after an overnight fast from both the groups (Control and Type-2 diabetic patients). Blood was collected in sterile tubes containing a mixture of potassium oxalate and sodium fluoride in the ratio of 3:1 and was centri-fuged at 3000rpm for 15minutes. The plasma obtained was used for the estimation of glucose and homocysteine levels. Blood sample was collected separately in EDTA vial (2ml) to determine HbA1c. Samples were stored at -20°C and used for various biochemical assays.

Biochemical Assays :-

1-Glucose : Blood glucose level was estimated using Enzymatic methods by (Rondo.United Kingdom Laboratories Ltd ,Co.Antrium.Kit .

2-Glycosylated : Haemoglobin(HbA1c): HbA1c was analyzed by using stanbio kit.

3- Homocysteine : The serum Hcy levels were estimated by enzymatic immunoassay method (EIA) using Axis-Homocysteine Enzyme Immunoassay (EIA) Kit from Axis-Shield Diagnostic ,UK.

Statistical Analysis

The data obtained was tabulated by using a SPSS-19 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 . A value of $p < 0.05$ was considered significant .

Results

The three groups for this study, thirty patients for all parameters (good glycemic control patients ,poor glycemic control patients and healthy control) were compared . Ages for (good glycemic control were 42.35 ± 5 ,poor glycemic control were 47.4 ± 10 and control 42.1 ± 5) and sex (good glycemic control :17 male and 13 female , poor glycemic control :18 male and 12 female and healthy control :15 male and 15 female) .These results were reported in table 1.Total HbA1c concentration in diabetes patients is high significant than healthy controls (7.8 ± 1 vs. 5 ± 0.57). Also HbA1c concentration in poor glycemic control is higher than good glycemic control (8.9 ± 0.71 vs. 6.6 ± 0.96).The results of total HbA1c in the subgroups with good and poor glycemic control are compared in figure 1 . As well homocysteine levels were significantly higher in diabetic patients compared to controls (25.2 ± 2.5 vs 12.1 ± 2.4 $\mu\text{mol/L}$, $p < 0.001$) 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 were not statistically significantly different in these subgroups .Each subgroup included the same of hyperhomocysteinemia (25.61 ± 2.3 vs. 24.48 ± 2.7 $\mu\text{mol/L}$ in patients with good and poor glycemic control respectively).

Table: 1 The results of (FBC ,BMI ,Age,HbA1c ,Hcy levels) in all patients

| Parameter | Good glycemic control n=30 | Poor glycemic control n=30 |
|--------------|-------------------------------|-------------------------------|
| Age | 42.35±5 | 47.4±10 |
| Fbc | 8.9±1.6 | 9.8±2.9 |
| BMI | 30.1±3.9 | 27.9±6.5 |
| HbA1c | 6.6±0.96 | 8.9±0.71 |
| Homocysteine | 25.61±2.3 | 24.48±2.7 |

mean±SD ,p<0.001

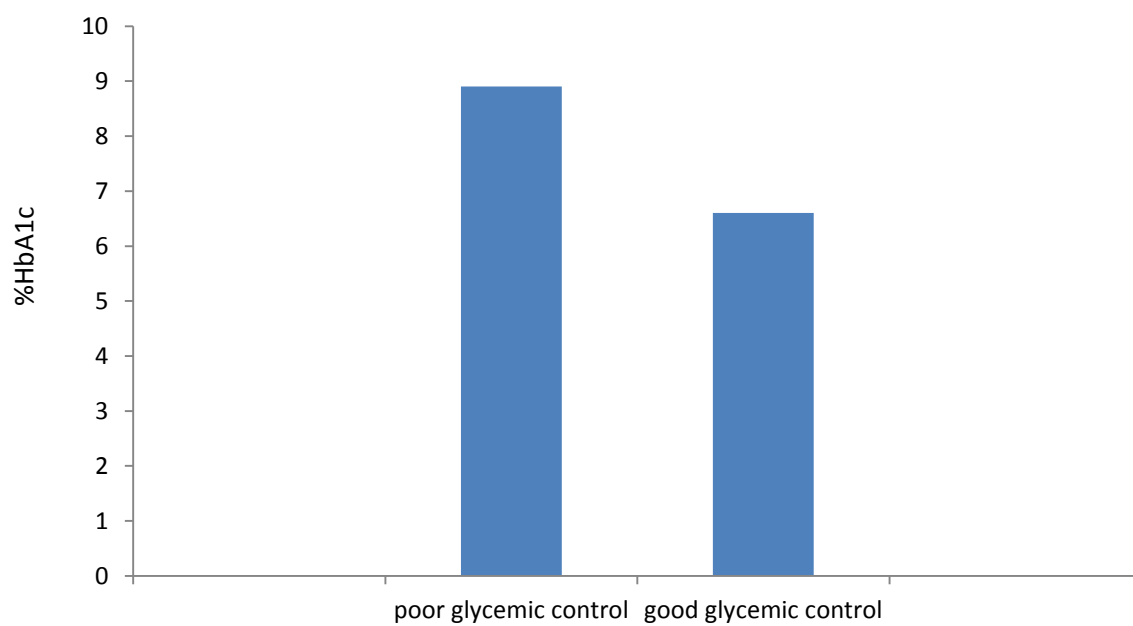


Figure 1. Total HbA1c in poor and good glycemic control patients .

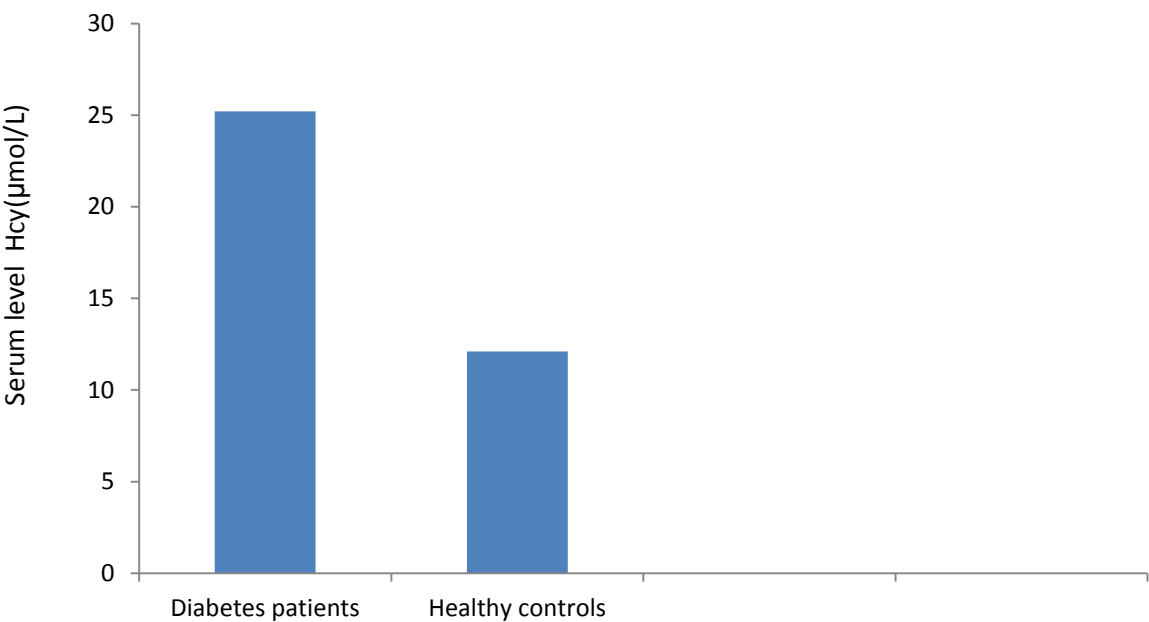


Figure 2. Total homocysteine in diabetes patients and healthy controls.

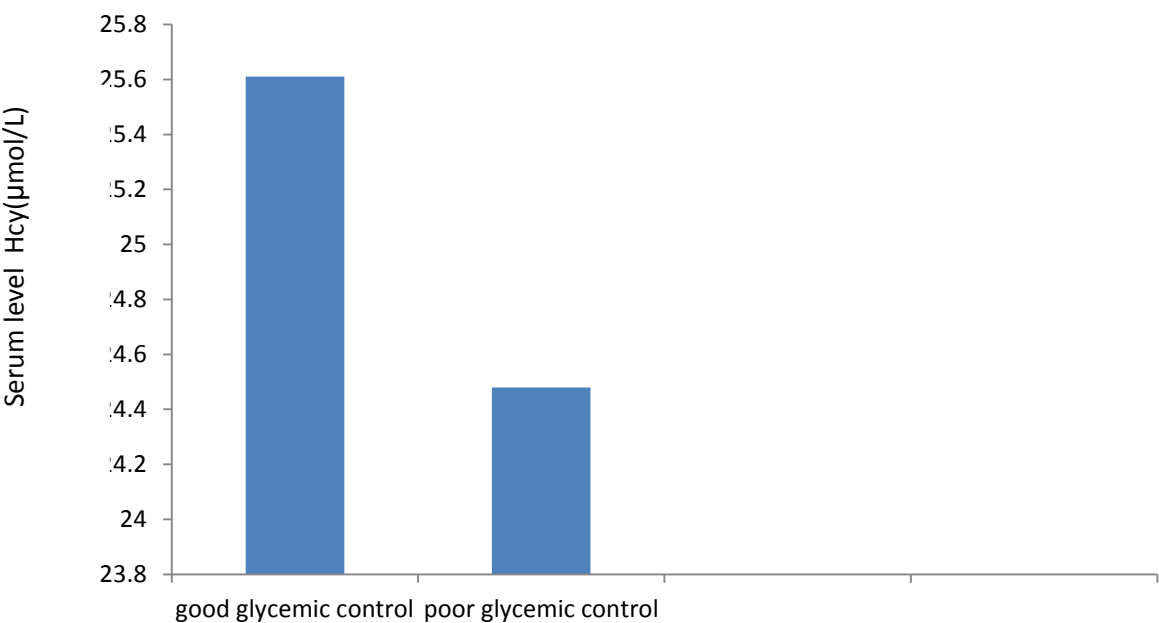


Figure 3. total homocysteine in good and poor glycemic control patients .

Discussion

Few reports to data have explored in prospective manner the role of glycemic control and homocysteine level along time .Therefore ,this study was concerned to assess the relationship between glycemic control and homocysteine level in patients with type 2 diabetes . The results of our study revealed significantly higher concentration of HbA1c and serum levels of homocysteine ,as well as a higher prevalence of hyperhomocysteinemia (serum homocysteine $>15 \mu\text{mol/L}$) in patients with type 2 Diabetes compared to controls(12).Our results coincide presence of mild hyperhomocysteinemia that play a role in development of cardiovascular disease in diabetic patient, as reported by some of previous studies (Al-Araji *et al.*,2012; angma *et al.*,2015 ; Singh and Singh,2011).

The observed elevation of homocysteine in diabetic patients could be attributed to insulin resistance (Letho *et al.*,2000 ; Basoglu,2011). Also long –term treatment with metformin may lead to deficiency of nutrient or micronutrient (folate, VB12 and B6)disrupt homocysteine metabolism and increases homocysteine level (Hoogeveen *et al.*,1997; Chen *et al.*,2012). Homocysteine levels may also affected by genetic factors (Trialists'Collaboration,2007) , demographic life style and various health factors (Rekha *et al.*,2012).

A significant finding in our study was although homocysteine level in diabetic patients is higher than controls but there is no significant differentiate between two sub groups ,this may be due either to an actual lack of such an association between the two factors or to the inconsistency of hyperglycemia during the progression of DM (Snieder *et al.*,2011). Besides ,evidence from both healthy and diabetic twins indicates that HbA1c levels are determined for a significant part by genetic factors .

This provides evidence that HbA1c is in part determined by factors other than glycemic control and may account for the variation in HbA1c levels among people (Cohen *et al.*,2006).

Some of previous studies have shown no significant correlation between serum homocysteine and glycemic control(Eslamipour *et al.*,2014; Aghamohammadi *et al.*,2011) .

Fotiou *et al.* reports there is no significant association between serum homocysteine and HbA1c concentration (Fotiou *et al.* ,2014) . In other study Zarnagh *et al.* investigate association of plasma level of total homocysteine with good and poor glycemic control in 100 Iranian patient with good and poor glycemic subgroup .He report there is no significant differentiate between two subgroups(Zarnagh *et al.*,2014) . They emphasized glycemic control don't influence plasma homocysteine level . Our findings are consistent with results of these studies . In other hand , there are some studies that glycemic control of diabetes may influence homocysteine levels (Goldstein *et al.*,2004). 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 .The result of this study showed there is significant positive correlation between metabolic control and homocysteine levels (Passaro *et al.*,2003). These authors have shown that patients with poor glycemic control of diabetes had significantly higher homocysteine levels in comparison to diabetics with normal HbA1c levels .

Although the former explanation of the results of our research , further follow –up or interventional studies will be necessary in order to increasingly clarify the significance of glycemic control on total homocysteine levels in diabetics .

In conclusion , these findings suggest glycemic control in type 2 DM was more strongly associated tHcy levels and these results are not enough for tHcy reduction, but there are several other factors that affect the tHcy levels , such as folate ,B12 ,B6 and some of them are yet unidentified .

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