Original Article

Effect of Glycemic Control on Homocystine Levels in Type 2 Diabetes Mellitus - A Six Month Follow-up Study

Zulfania[1], Soheb Rehman[2] and Tahir Gaffar[3]

ABSTRACT

Objective: Review of the available reports is unable to determine with any certainty the association of glycemic control (HbA1c) and homocystine (Hcy) in diabetes. Therefore, the present study was carried out to ascertain the relation of glycemic control with Hcy levels.

Study Design: Cohort study

Place and Duration of Study: This study was conducted at the Endocrinology Unit of Hayatabad Medical Complex (HMC). A laboratory analysis was done in the laboratory of Rehman Medical Institute (RMI), Peshawar from April 2015 to October 2015.

Materials and Methods: This cohort study was conducted on 125 patients who were known type 2 diabetics visiting Endocrinology unit of HMC. A detailed medical history and clinical examination was carried out to exclude co morbidities. At all three visits i.e baseline, first and second follow up HbA1c and Hcy were recorded. Statistical analysis of the data was done by SPSS version 20 using Pearson correlation test to correlate HbA1c levels with Hcy levels.

Results: The mean age of 125 study subjects was 51± 8.37 years,out of these 68% were females and 32% were males. The mean HbA1clevels reduced from baseline (9.64±2.25%) to (8.56±1.99%) till second follow up. Mean Hcy levels did not dropped from baseline (10.04±4.31) to second follow up (11.46±3.95), rather increased. There was no correlation of Hcy with HbA1c in baseline data (r=-0.052, p=0.576), first follow up (r=-0.023, p=0.836) and second follow up (0.098, p=0.521). No correlation was also evident in change of Hcy and HbA1c levels noticed from baseline till second follow up.

Conclusion: The study concluded that there is no correlation of Hcy and HbA1c in baseline as well as follow up data.

Key Words: HbA1c, glycemic control, , Hcy, Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels which may result from defective insulin secretion, its action or both. Due to long term hyperglycemia, this disease causes damage and dysfunction of organs like heart, kidneys, eyes, blood vessels and nerves[1].

WHO survey conducted in 1995 showed that Pakistan was at number 8th position in top ten countries with high diabetic prevalence, with about 4.3 million people suffering from diabetes mellitus. This survey also anticipated that in year 2025, Pakistan will be on the 4thposition with 14.5 million people having diabetes[2].

1. Department of Physiology / Biochemistry[2], Rehman Medical College, Peshawar.
3. Department of Endocrinology, Lady Reading Hospital, Peshawar.

Correspondence: Dr. Zulfania, Assistant Professor of Physiology, Rehman Medical College, Peshawar.
Contact No: 0332-9297758
Email: Zulfania.khan@rmi.edu.pk

A Pakistani survey conducted in 2012 showed the prevalence of diabetes as 7.7% in rural and 10.6% in urban areas[3]. An international study reported that about 7 million people were suffering from diabetes in Pakistan in year 2016[4].

Homocysteine (Hcy), formed during methionine demethylation in all cells of body, is a sulfur-containing, small, non-protein-forming amino acid. Extra amount of intracellular Hcy is exported to the circulation. In circulation approximately 1% remains in free form and the remainder binds to albumin or forms disulfide dimers, principally with cysteine[5].An elevated Hcy level is considered as a risk factor for atherosclerosis, cerebrovascular disease, and peripheral vascular disease. The causes of hyper-homocysteinemia include genetic enzymes deficiencies, deficiency of vitamins (folic acid, B_{12}, B_6), certain medications, and impaired renal function[6].

A study suggested that Hcy influences endothelial function by enhancing aprothrombotic environment causing platelet activation and endothelial leukocyte interactions[7]. In addition, Hcy enhances inflammatory responses that are recognized for their role in atherosclerotic disease[8]. Early prediction of

cardiovascular events by elevated plasma Hcy is reported by different authors. Studies done in Nigerian-Africans showed a moderate rise in plasma Hcy in cases of established cardiovascular disease[9].

Studies of Hcy in type 2 diabetics have shown variable and uncertain results; some of them have revealed a positive association of Hcy levels with glycemic control[10] but Hoogeven etal in their study found no correlation between the two[11]. Therefore, the present study was carried out to ascertain the relation of glycemic control with Hcy.

MATERIALS AND METHODS

This cohort study was conducted in Endocrinology Unit of Hayatabad Medical complex (HMC). Laboratory analyses of the collected samples were done in the laboratories of Rehman Medical Institute (RMI), Peshawar from April 2015 to October 2015. Type 2 diabetics with age 45-65 years admitted in Endocrinology Unit of HMC were included in this study. All subjects who had type 1 diabetes mellitus, any acute infection or chronic inflammatory disease like infection of upper or lower respiratory tract, urogenital tract, GIT were also not included..Moreover patients with anemia or taking NSAIDS, lipid lowering drugs or are pregnant or breast feeding were excluded.

Data Collection: A detailed medical history and physical examination was conducted on the subjects. All data were recorded on pre-designed Performa (Annex A). A fasting blood sample was taken by venipuncture and was kept in ice packs until transferred to Rehman medical institute laboratory where it was centrifuged and stored at -80°C.Levels of Hcy was measured by micro particle enzyme immunoassay and HbA1c by immunoassay by using (Abbott) laboratory kits according to the protocol.

Data Analysis: Data about age, gender, BMI, fasting blood glucose, HbA1c and Hcy was entered into SPSS on daily basis. Data was analyzed to measure the frequency proportions, percentages, ratios, means and standard deviations. Pearson's correlation coefficient was used to determine the correlation between Hcy and HbA1c.

RESULTS

In this study, 125 type 2 diabetics were examined and followed up for 6 months. The initial blood samples were analyzed to measure HbA1c and Hcy. Then samples were taken again taken after three and six month's duration of the initial samples. Due to loss of data in follow up only about 70% of the calculated sample size (110 patients) could be used for final analysis

In Table No. 1, HbA1c0 indicates baseline glycosylated hemoglobin level, HbA1c1 indicates first follow up and HbA1c2 indicates glycosylated hemoglobin levels of second follow up in percentage (%).Table 3.2 shows

mean baseline glycemic control (HbA1c0) was 9.64 ± 2.25 %. The mean glycaemic control at 1st follow up (HbA1c1) decreased to 8.83 ± 2.01 %. The mean glycaemic control in 2nd follow up (HbA1c2) further reduced to 8.56 ± 1.99 %. The change of HbA1c level in baseline and first follow up, and that between baseline and second follow up was significant. While the HbA1c change during first and second follow up was not significant.

Table No.2 shows mean baseline homocysteine levels (Hcy0) was $10.04 \pm 4.31 \mu mol/l$. The mean Hcy level in first follow up (Hcy1) was 10.84 ± 4.18 $\mu mol/l$. The mean Hcy level in second follow up (Hcy2) was $11.46 \pm 3.95 \mu mol/l$. The change of Hcy level in baseline and first follow up was insignificant, however change of Hcy levels between first and second follow up and baseline and second follow up was significant.

Figure 1 shows no correlation of HbA1c0 and Hcy0 (with $r = -0.052$ P-Value = 0.576). Figure 2 shows no correlation of HbA1c1 and Hcy1 (with $r = -0.023$ P-Value = 0.836). Figure 3 shows no correlation of HbA1c2 and Hcy2 in 2nd follow up (with $r = 0.098$ and p = 0.521).

Table No.1: Baseline, 1st follow up and 2nd follow up HbA1c levels

Sr.#	Varia-bles	Mean (%)	Standard Deviation	95%CI	p value
1	HbA1c0	9.64	2.25	-1.491 to -0.132	0.020
	HbA1c1	8.83	2.01		
2	HbA1c1	8.83	2.01	-0.362 to 0.910	0.396
	HbA1c2	8.56	1.99		
3	HbA1c2	8.56	1.99	0.409 to 1.762	0.002
	HbA1c0	9.64	2.25		

Table No.2: Baseline, 1st and 2nd follow up Hcy

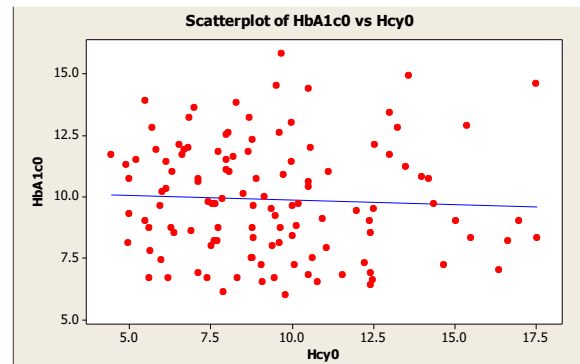| Sr. #	Varia-bles	Mean (mg/l)	Standard Deviation	95%CI	p value
1	Hcy0	10.04	4.31	-1.153 to 1.551	0.772
	Hcy1	10.84	4.18		
2	Hcy1	10.84	4.18	-2.909 to -0.322	0.015
	Hcy2	11.46	3.95		
3	Hcy2	11.46	3.95	-2.733 to -0.100	0.035
	Hcy0	10.04	4.31		

Scatterplot of HbA1c0 vs Hcy0

Figure No.1: Correlation of HbA1c0 and Hcy0 in baselinedata (r = -0.052, P-Value = 0.576).
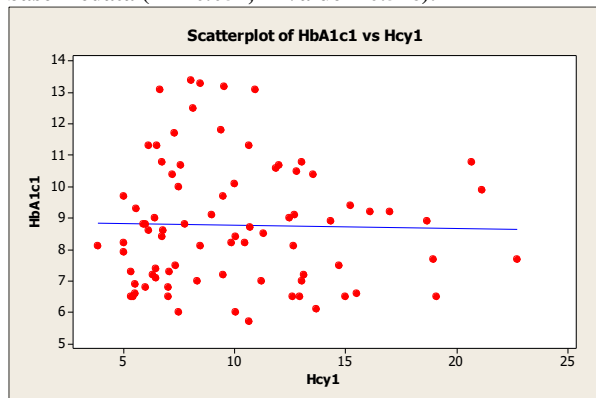

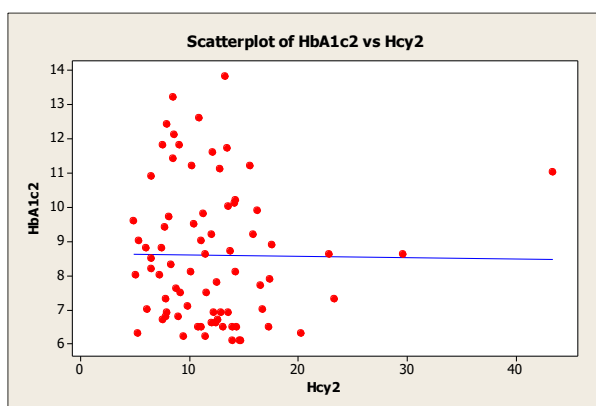**Figure No.2: Correlation of HbA1c1 and Hcy1 in first follow updata (r = -0.023, P-Value = 0.836).**

Figure No.3: Correlation of HbA1c2 and Hcy2 in 2nd follow up(r= 0.098, P-Value = 0.521)

DISCUSSION

In the present study the mean HbA1c levels dropped from 9.64% to 8.56% significantly till the 2nd follow up. Similar drop in HbA1c level was reported in studies in which type 2 diabetics were followed up for four months or more months[13,14].

In the current study the mean Hcy level observed in baseline sample increased in first and second follows up. Similar rise in Hcy levels was observed in a study in which lipid profile and cardiovascular risk markers were checked in type 2 diabetics[15]. The possible reason for this mild rise in Hcy levels might be that dose of anti-diabetic medications were adjusted to control HbA1c levels in subsequent follow ups as most of the patients were receiving metformin as monotherapy or in combination with insulin and other hypoglycemic drugs, it could have led to decreased vitamin B12 level in their bodies resulting in slight rise of Hcy levels. Passaro et.al. reported a fall in Hcy levels in type 2 diabetics when followed for 3 years[16] but most of the subjects were on anti-diabetic medications other than metformin in their study. The Hcy level of $9.66 \pm 3.23 \mu mol/l$ in type 2 diabetics without cardiovascular complications was reported by Tarkun et.al. is in agreement with the result of present[17]. As

Hcy levels remain in normal range in patients without cardiovascular and renal complications that's why the present study with participants without diabetic complications reported normal Hcy levels.

The present study revealed no correlation of Hcy and HbA1c in baseline data, first follow up and second follow up. Similarly there was no correlation of the change of Hcy and HbA1c levels in all three phases. These findings endorse the report of other studies including a study conducted in Iran in which healthy controls were compared with type 2 diabetics having either good or poor glycemic control. They observed that although Hcy levels were higher in diabetics but not significantly different among various groups. They concluded that glycemic control does not influence Hcy levels and no correlation exists between the two variables[18]. Other researchers like Hoogeven et al. also found no association of glycemic control and Hcy[19]. A study in which Aghamohammadi et al. studied the correlation of Hcy and HbA1c in 70 type 2 diabetic males reported no statistically significant association between the two variables[20]. This study also had the same conclusion that just keeping glycemic control is not sufficient for maintaining lower Hcy levels but other measures such as use of vitamin B12 and folic acid is also necessary for diabetics. The effect of improved glycemic control and insulin sensitivity on Hcy levels was investigated by Pouwels et al. and they also confirmed that HbA1c levels have no influence on Hcy[21]. A study conducted on Kenyan type 2 diabetics without any cardiovascular disease also showed no effect of HbA1c levels on Hcy[22].

On the other hand significant correlation of Hcy with HbA1c was noticed by Passaro et al. who did 3 years follow up of type 2 diabetics. These researchers noticed that a significant positive correlation exists between glycemic control and Hcy[16]. These findings might be because of longer follow up duration of the study necessary to achieve the glycemic control required to influence Hcy levels and its correlation. A very weak positive and statistically insignificant correlation of HbA1c and Hcy was reported in a study of type 2 diabetics who didn't have any cardiovascular disease[17]. A study done to assess the nutritional status and Hcy levels of elderly type 2 diabetics, reported a very weak association of HbA1c and Hcy[23]. Therefore, it can be inferred that the correlation of Hcy with HbA1c is not certain from researcher's point of view and further studies of larger sample size and longer duration must be conducted to ascertain the association between the two variables.

The limitations of this study was a small sample size (n=125) and loss of 39% of the baseline data in the follow up which accounted for 30% of sample size (n=110). Only 70% data was available for final analysis which is a common impediment of conducting prospective studies in uneducated and poor population coming to public sector hospitals for consultation.

CONCLUSION

The association of Hcy and HbA1c could not be ascertained by the present study, but it may be mentioned that longer period of glycemic control may be required to influence the Hcy level to extent that a significant correlation is established.

Author's Contribution:

Concept & Design of Study: Zulfania
Drafting: Zulfania, Soheb Rehman
Data Analysis: Tahir Gaffar
Revisiting Critically: Soheb Rehman
Final Approval of version: Zulfania

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes Mellitus. Diabetes Care 2013;36(Suppl 1):S67-74.
2. World Health Report. World health Organization (WHO).1211;Geneva 27, Switzerland, 2003.
3. Ramachandran A, Snehalatha C, Shetty A, Nanditha A. Trends in prevalence of diabetes in Asian countries. World J Diabetes 2012;3(6):110.
4. Sandu M-M, Protasiewicz DC, Firanescu AG. Data regarding the prevalence and incidence of diabetes mellitus and prediabetes. Romanian J Diabetes Nutr Metabol Dis 2016;23(1):95-103.
5. FruchartJC, Nierman MC, Stroes ES, Kastelein JJ, Duriez P. New risk factors for atherosclerosis and patient risk assessment. Circulation 2004;109 (suppl):III-15– III-19.
6. Cheng Z, Yang X, Wang H. Hyperhomo-cysteinemia and Endothelial Dysfunction. Curr Hypertens Rev 2009;5(2):158–65.
7. Pasterkamp G, Algra A, Grobbee DE, Banga JD, van der Graaf Y. Homocysteine and the stage of atherosclerotic disease: A study in patients suffering from clinically silent and clinically manifest atherosclerotic disease. Eur J Clin Invest 2002;32:309–15.
8. Poddar R, Sivasubramanian N, Dibello PM, Robinson K, Jacobson D. Homocysteine induces expression and secretion of monocyte chemo attractant protein-1 and interleukin-8 in human aortic endothelial cells: Implication for vascular disease. Circulation 2001;103:2717–23.
9. Ebesunun MO, Agbedana EO, Taylor GOL, Oladapo OO. Plasma lipoprotein (a), homocysteine and other cardiovascular disease(CVD) risk factors in Nigerians with established CVD. Appl Physiol Nutr Metab 2008;33:282–9.
10. Shaikh MK, Devrajani BR, Shaikh A, Shah SZA, Shaikh S, Singh D. Plasma Homocysteine Level in Patients with Diabetes mellitus. World Applied Sciences J 2012;16(9):1269-73.
11. Hoogeven EK, Kostense PJ, Beks PJ, et al. Hyperhomocysteinemia Increases Risk of death Especially in type 2 Diabetes. Circulation 2000; 101: 1506-10.
12. Huang T, Asimi S, Lou D, Li D. Plasma phospholipid polyunsaturated fatty acids and homocysteine in Chinese type 2 diabetes patients. Asia Pac J Clin Nutr 2012;21(3):394-9.
13. Khatana SAM, Taveira TH, Choudhary G, Eaton CB. Change in Hemoglobin A1c and C-Reactive Protein Levels in Patients With Diabetes Mellitus. J Cardiometabolic Syndrome 2009;4(2):76-80.
14. Siaw MYL, Chew DEK, Dalan R, Abdul Shakoor SAKK, Othman N, Choo CH, et al. Evaluating the Effect of Ramadan Fasting on Muslim Patients with Diabetes in relation to Use of Medication and Lifestyle Patterns: A Prospective Study. Int J Endocrinol 2014.
15. Group TS. Lipid and Inflammatory Cardiovascular Risk Worsens Over 3 Years in Youth With Type 2 Diabetes The today clinical trial. Diabetes Care 2013;36(6):1758-64.
16. Passaro A, Calzoni F, Volpato S, Nora ED, Pareschi PL, Zamboni PF, et al. Effect of metabolic control on homocysteine levels in type 2 diabetic patients: a 3 year follow up. J Int Med 2003;254(3):264-71.
17. Tarkun I, Arslan BC, Canturk Z, Tarkun P, Kozdag G, Topsever P. Homocysteine concentrations in type 2 diabetes mellitus patients without cardiovascular disease: relationship to metabolic parameters and diabetic complications. Turkish J Endocrinol Metabolism 2003;1:11-7.
18. Heydari-Zarnagh H, Nejat Shookohi A, Nourozi A. Effect of Glycemic Control on Homocysteine Levels in Type 2 Diabetic Patients without Cardiovascular Disease. Zahedan J Res Med Sci 2014;16(1):23-7.
19. Hoogeveen EK, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ, et al. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes 5-year follow-up of the Hoorn Study. Circulation 2000;101(13):1506-11.
20. Aghamohammadi V, Pourghassem Gargari B, Aliasgharzadeh A. Evaluation of the level of plasma homocysteine in patients with type 2 diabetes mellitus under metformin treatment and its correlation with serum total antioxidant capacity, malondialdehyde, creatinine, insulin resistance and glycemic control. ZUMS J 2011;19(76):1-10.
21. Pouwels M-JJ, den Heijer M, Blom HJ, Tack CJ, Hermus AR. Improved insulin sensitivity and metabolic control in type 2 diabetes does not influence plasma homocysteine. Diabetes Care 2003;26(5):1637-9.
22. Otieno CF, Vaghela V, Ogola EN, Amayo EO. Patterns of homocysteine in Kenyans with type 2 diabetes without overt cardiovascular disease at Kenyatta National Hospital, Nairobi. East African Med J 2006;82(12).
23. Chen SF, Cui CL, Wu P, Xie NZ. Relationship of serum homocysteine level with nutritional status and HbA1c level in elderly inpatients. Int J Clin Exp Med 2013;6(9):779-84.