Evaluation of serum levels of homocysteine, C-peptide and lipid profile in type I and type II diabetic patients in Hawler province/ Iraq

Received: 17/10/2011 Accepted: 29/7/2012

Abdulrahman J. Mohammed * Abdulkader A. Alnakshabandi * Abdulrahman Al-Bazzaz **

Abstract

Background and objective: Diabetes mellitus is associated with many complications such as cardiovascular disease, even in the presence of intensive glycemic control. Homocystein takes part in the development of atherosclerosis and vascular injury and it is suggested to contribute in atherosclerotic process of diabetes mellitus. C-peptide recently used to diagnose diabetes.The present study was designed to evaluate serum levels of Homocysteine, C-peptide levels and lipid profile in diabetic patients.

Methods: This study includes 75 diabetic patients (35 type 1 and 40 type 2) and 30 controls. After 12 hours fasting, serum homocystiene, C-peptide, lipid profile, HbA1c and blood glucose were measured for patients and controls.

Results: The results showed that homocysteine level in type 1 and type 2 diabetic patients significantly increased as compared with controls. And the C-peptide level in type 1 diabetic patient was very low, while in type 2 diabetic patients and in controls was normal. The results also showed that the lipid profiles (except HDL) were increased in diabetic patients.

Conclusion: Serum homocysteine (Hcy) level and lipid profile (except HDL) are significantly elevated in diabetic patients. The C-peptide level in type 1 diabetic patient was very low, while in type 2 and controls was normal.

Keywords: Diabetes mellitus, Homocysteine, C-peptide, Lipid profile.

Introduction

Diabetes mellitus is a condition in which the body either does not produce enough, or does not properly respond to, insulin, a hormone produced in the pancreas, and this causes glucose to accumulate in the blood [1,2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action [3].

Homocysteine: Homocysteine is a nonessential sulphur-containing amino acid and an intermediary metabolic product derived from the demethylated essential amino acid methionine [4]. Circulating homocysteine derives from the interplay of genetic and environmental factors involved in the homocysteine/methionine metabolic cycle. Ageing and gender, renal function, the status of nutritional coenzymes of vitamin B12, B6 and folate, together with lifestyle factors such as smoking, are known determinants of plasma homocysteine concentration in the general population, also individual genetic background may predispose to hyperhomocysteinemia [5,6]. During the past few years, elevated blood levels of homocysteine have been linked to increased risk of premature coronary artery disease, stroke, and thromboembolism [7]. The mechanism of Hcy-mediated vascular injury involves oxidative damage,since Hcy can undergo autoxidation in the plasma or

* Department of Clinical Analysis, College of Pharmacy, Hawler Medical University, Erbil, Iraq.
** Department of Biochemistry, Faculty of Pharmacy, Al-Ahliyya Amman University.

intracellularly to form various reactive oxygen species [8]. Average fasting plasma total Hcy levels for healthy human subjects range between 6 and 12 µmol/L [9]. There are several studies conducted to evaluate the presence of hyperhomocysteinaemia and its consequences in diabetic patients. Although hyperhomocysteinaemia is determined as an independent and a graded risk factor for the development of cardiovascular disease, the clinical outcome of hyperhomocysteinaemia and/or the presence of factors leading to hyperhomocysteinaemia in diabetic patients is still unclear [3] . Hyperhomocysteinaemia is known to be associated with atherosclerosis, and this association is stronger in individuals with diabetes than in nondiabetic subjects [10].

C-peptide: is a peptide that is made when proinsulin is split into insulin and C-peptide. It splits before proinsulin is released from endocytic vesicles within the pancreas, one C-peptide for each insulin molecule [3]. C-peptide is often used in the clinic to monitor the beta cell function in the diabetic patient and to differentiate between type 1 and type 2 diabetes [11] . The pancreas of patients with type 1 diabetes is unable to produce insulin and therefore they will usually have a decreased level of C-peptide and insulin levels, whereas C-peptide and insulin levels in type 2 patients are normal or higher than normal[12,13]. C-peptide concentrations are considered a more reliable reflection of pancreatic beta cell function (and hence insulin secretion) than insulin concentrations, because the half life of C-peptide in the circulation is two to five times longer than that of insulin. Fasting serum concentrations of C-peptide in healthy people range from 0.5 to 2 ng/ml [14]. Dyslipidemia are common in diabetes and contribute significantly to its complications. Its alteration in lipid metabolism increased cardiovascular risk, and so the mortality and morbidity among diabetic patients [15]. The aim of the study to evaluate the blood levels of homocysteine, C-peptide, lipid profile and glucose and the metabolic control measured by HbAC, in type 1 and type 2 diabetic patients as compared with controls.

Methods

Subjects: This study was carried out at the Diabetic Center, in Hawler city, during the period from November 2009 to March 2010, it included 75 diabetic patients (40 type 2 and 35 type 1) and 30 normal subjects as controls. Out of the 35 type 1 diabetic patients, 17 are male and 18 are females, their ages ranged between 13-28 years (mean 24.8±5.5 year).While the other 40 having type 2 diabetes (12 are males and 28 are females), their ages ranged between 36-59 years (mean 49.1±10.4 year), 30 normal subject were included as a control group. Their ages ranged between 23-51 years (39.5±8.72).10 ml of samples venous blood was drawn from each fasting (10-12 hours) patients and controls, using sterile disposable syringe. 3 ml of the blood was transferred to disposable EDTA containing plain tube, and utilized for determination of HbA1c. The remaining 7 ml of the blood was transferred to disposable plain tube and let stand for 30 minutes to clot. The serum was separated by centrifugation at 3000 rpm for 5 minutes, and collected in plain tube and kept frozen. The serum was utilized for determination of (Glucose, Homocysteine, C-peptide, cholesterol, triglyceride, HDL, LDL and VLDL).

Methods: Homocysteine and C-peptide were measured by a commercial ELISA kit and the lipid profile also measured by their specific kits, according to manufactural procedure.

Results

The results showed that all the diabetic patients showed significantly higher serum fasting sugar in conjunction with HbA1c versus their control pend. As all the diabetic patients under current study had HbA1c more than 8% so they are all poorly controlled. Serum homocysteine levels in type 1 and type 2 diabetic patients are

Table 1: laboratory investigations for patients and healthy control group

		N	Mean	Std. Deviation	P	Significacne
Homocysteine	Control	30	7.6300	2.13766	0.004	AxB*
	Type 1	35	13.8343	7.06382	0.004	AxC*
	Type 2	40	13.7550	8.66523		BxC
C-peptide	Control	30	1.0780	0.50346	<0.001	AxB*
	Type 1	35	0.3954	0.26983	<0.001	AxC*
	Type 2	40	0.7410	0.31141	<0.001	BxC*
HbA1c	Control	30	5.6850	0.84310	<0.001	AxB*
	Type 1	35	10.2229	2.18189	<0.001	AxC*
	Type 2	40	8.9300	2.08858		BxC
FSG	Control	30	108.3500	17.53875	<0.001	AxB*
	Type 1	35	239.0286	75.18310	<0.001	AxC*
	Type 2	40	186.2750	53.79209	0.003	BxC*
Cholesterol	Control	30	164.8000	26.01740	<0.001	AxB*
	Type 1	35	228.6857	78.25703	<0.001	AxC*
	Type 2	40	242.1000	85.21972		BxC
TG	Control	30	138.0500	34.68880		AxB
	Type 1	35	182.8286	110.88295	0.008	AxC*
	Type 2	40	219.6250	98.03314		BxC
HDL-c	Control	30	55.0000	8.40426		AxB
	Type 1	35	53.8286	10.71416		AxC
	Type 2	40	49.1000	8.26423		BxC
LDL-c	Control	30	82.4000	28.29246	0.001	AxB*
	Type 1	35	138.2857	68.89104	0.001	AxC*
	Type 2	40	149.1350	75.76056		BxC
VLDL-c	Control	30	27.4000	7.00676		AxB
	Type 1	35	36.5714	22.10574	0.007	AxC*
	Type 2	40	43.8750	19.62754		BxC

A: control, B: type 1, C: type 2, (*): significance. P value less than 0.05 considered as statistically significant

significantly increased when compared with their levels in controls. Serum C-peptide levels in type 1and type 2 diabetic patients' showed significantly low concentrations as compared with controls or between themselves although its concentration in type 2 diabetic patients is within normal range. The lipid profile showed that the levels of (cholesterol, triglyceride, LDL and VLDL) are increased in diabetic patients when compared with controls, with different significances, while HDL remained within normal range.

Discussion

Many past published studies dealt with plasma Hcy levels in both Type 1 and Type 2 diabetes had yielded a multiplicity of results. The present study showed that homocysteine level in type 1 and type 2 diabetic patients significantly increased as compared with its level in controls. This result agreed with results of Hultberg *et al* [16] and Hofmann *et al* [17], in which they showed that diabetic patients had a significantly increased concentrations of total plasma homocysteine level. It also agrees with studies by Colwell [18] and Becker *et al* [19] who showed that increased homocysteine levels predicteding the risk factor for vascular complications in diabetic patients. other studies, on the other hand, were in disagreement with this result mushi *et al* [20] and Drzewoski *et al* [21] , who they found that fasting homocysteine levels were found to be similar in diabetic patients and healthy controls. In another studies conducted by Fonseca *et al* [22] and Tutunco *et al* [4] revealing normal fasting homocysteine levels in the diabetic population with various complications. This difference in the results may reflect the many mechanisms involved in the regulatory process of plasma homocysteine. Circulating homocysteine derives from the interplay of genetic and environmental factors involved in the homocysteine/methionine metabolic cycle. Ageing and gender, renal function, the status of nutritional vitamin coenzymes B12, B6 and folate, together with lifestyle factors, are known determinants of plasma homocysteine concentration in the general population, also individual genetic background may predispose to hyperhomocysteinemia[5,6]. Hyperglycemia causes abnormal carbohydrates, lipids and proteins metabolisms which may leads to abnormal elevated homocysteine levels also medications like metformin which is used in great number of diabetics is known to cause vitamins B12 and folate deficiencies that causes consequently leads to hyperhomocysteinemia [23] . This study showed also that the mean serum C-peptide levels in type 1 and type 2 diabetic patients are decreasing significantly as compared with their controls. In type 1 it is significantly low as compared to type 2 diabetic patients although the C-peptide level is within normal range in type 2 diabetic patients and in controls. This result is in agreement with results of studies conducted by [12,13], they showed that the patients with type 1 diabetes usually have a decreased level of C-peptide , whereas C-peptide level in type 2 diabetic patients was normal or higher than normal . This is because in pancreas of patients with type 1 diabetes the function of beta cell is drastically reduced or totally absent shortly after the onset of the disease so it is unable to produce insulin and therefore they usually have a decreased level of C-peptide and insulin levels since these two components are secreted in equimolar amounts , while the pancreatic beta cell in insulin resistant type 2 diabetic patient functions normally, therefore it produce insulin normally, so they usually have normal level of C-peptide and insulin, or some time higher than normal because the resistance to insulin in type 2 diabetic patient well cause the pancreas to produce excess amount of insulin [12,13].

Conclusion

The data of this study showed that:-

1. Serum homocysteine levels significantly elevated in diabetic patients, which suggests that Hcy might play role in the pathogenesis of vascular complications in individuals with diabetes mellitus.

2. Increased levels of cholesterol, triglycerides, VLDL and LDL-C showed that diabetic patients may present a high risk of atherosclerosis and vascular complications of diabetes mellitus.

3. It is better for newly diagnosed diabetic patients to measure their C-peptide levels as a marker for distinguishing between type 1 and type 2 diabetes, since some of the type 1 patients misdiagnosed as type 2 .

References

1. Tierney LM, Mcphee S, Papadakis MA. Current medical diagnosis and treatment. 7th edition. New York: Lange Medical books MC Graw-Hill; p. 1203-15. (2007).
2. Rother KI. Diabetes treatment bridging the divide. The New England Journal of Medicine (2007) ; 356: 1499-501.
3. David C, John D, Daniel D, Glenn D, Marcelle I, et al. Greenspans basic and clinical endocrinology.5th ed. New York: Lange Medical books MC Graw-Hill; (2007).
4. Tutunco N, Erbas T, Alikasifoglu M, Tuncblec E. Thermolabile Methylene tetrahydrofolate reductase enzyme genotype is frequent in type 2 diabetic patients with normal fasting homocysteine level. Journal of Internal Medicine (2005) ; 257: 446-53.
5. Nygard O, Refsum H, Uland PM. Coffee consumption and plasma total homocysteine study. AMJ Clin Nutr 1997; 65: 136-43.
6. Hayden M, Tygagi S. Is type 2 diabetes mellitus is a vascular disease with hyperglycemia a late manifestation. Cardiovasc Diabetole (2003) ; 2:2.
7. Verhoef P. Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. Vascular biology 1997 ; 17: 989-95.
8. Wijekoon E, Brosnan M, Brosnan T. Homocysteine metabolism in diabetes. Metabolism 2007 ; 35: 1175-77.
9. Allone N, Andrew G, Jacob S, Irwin H. The kidney and homocysteine metabolism. J Am Soc Nephrol 2001 ; 12: 2181-89.
10. Smulder Y, Racki M, Slaats E. Fasting and post methionine homocysteine level in non insulin dependent diabetes mellitus: determinant and correlation with retinopathy, albumin urea and cardiovascular disease. Diabetes Care 1999 ; 22: 125-32.
11. Marques G, Fontaine M, Roger J .C-peptide: much more than a by product of insulin biosynthesis. Pancreas 2004 ; 29: 231-8.
12. Wickamasinghe L, Chazan B, Farrow M, Bansal S, Basso S. C-peptide response to oral glucose and its clinical role in elderly. Oxford Journals 1994 ; 21: 103-8.
13. Carina T, Mona L, Bengt S. Predictability of C-peptide for autoimmune diabetes in young adult diabetic patients. Pract Diab Int 2001 ; 18: 3-5.
14. David B . Carbohydrates. In:Carl A, Edward R, David E.editors. Tietz fundamentals of clinical chemistry.6th ed. United States of America: Saunders Elsevier (2008).
15. Nucitarhan S, Ozben T, Tuncer N. Serum and urine malandialdehyde levels in NIDDM patients with and without hyperlipidemia. Free Radc Biol Med 1995 ; 19: 893-6.
16. Hultberg B, Agardh C, Agardh E, Ardian M . Poor metabolic control early age onset and marginal folate deficiency are associated with increasing level of plasma homocysteine in insulin dependent diabetes mellitus. Scand J Clin Lab Invest 1997; 57: 595-600
17. Hofmann M, Kohl B, Zumbach M. Hyper homocysteinemia and endothelial dysfunction in insulin dependent diabetes mellitus. Diabetic Care 1998 ; 21: 841- 8.
18. Colwell JA. Elevated plasma homocysteine and diabetic vascular disease. Diabetes care 1997; 20: 1805-6.
19. Becker P, Kostense J, Bos G, Heine J. Hyperhomocysteinaemia is associated with coronary events in diabetes. J of Intern Med 2003; 253: 293-300.
20. Mushi M, Stone A, Finik B, Fonseca V. Hyper homocysteine following a methionine load in patients with non insulin dependent diabetes mellitus and macrovascular disease. Metabolism 1996 ; 45: 133-5.
21. Drzewoski J, Czupryniak L, Chwatko G, Bald E. Total plasma homocysteine and insulin level in type 2 diabetic patients with secondary failure to oral agent. Diabetes Care 1999 ; 22:2097-9.
22. Fonseca V, Mudaliar S, Schimit B, Fink L, Kern P, Henry R. Plasma homocysteine concentrations are regulated by acute hyper insulinemia in non diabetic but not type 2 diabetic patient. Metabolism 1998; 47: 686-9.
23. Champe P, Harvey R. Biochemistry. Lippincots illustrated review. 4th ed. New York: Lippincott Williams and Wilkins. 2008: p 416-20.