Iranian Journal of Pathology (2012)7 (1), 14 - 18

Original Article

Reduced Serum Homocysteine Levels in Diabetic Patients

Forough Kajbaf[1,2], Mohammad Ali Ghaffari[2,3], Mohammad Javad Kajbaf[2,4]

1. Dept. of Physiology, Islamic Azad University, Qom, Iran

2. Razi Clinical Lab., Ahwaz, Iran

3. Dept. of Biochemistry, Jondishapour University of Medical Sciences, Ahwaz, Iran

4. Dept. of Microbiology, Jondishapour University of Medical Sciences, Ahwaz, Iran

ABSTRACT

Background and Objectives: Type 2 diabetes is the most prevalent form of diabetes mellitus and is associated with a variety of complications. Homocysteine is an important independent risk factor for atherosclerotic diseases in both diabetic and non diabetic subjects. The association between these two is still unclear. The aim of this study was to assess the serum homocysteine levels in uncomplicated type 2 diabetic patients and control subjects.

Materials and Methods: Eighty five diabetic patients and 85 healthy control subjects with the mean age of 57.65 and 57.68 years, respectively, were selected during 2010 in Ahwaz City, southwest of Iran. Serum glucose, lipids and lipoproteins were measured by standard enzymatic techniques and homocysteine levels by enzyme –linked immunosorbent assay method.

Results: In patients as a whole and with respect to the gender, homocysteine levels were generally lower than controls. Compared to other studies, homocysteine levels ranging from 12.19 to 18 µmol/l were slightly higher in both population.

Conclusion: Homocysteine levels, however, were compatible with normal range of adults. The patients were not nephropathic and it is most likely that this is the main reason for maintaining the normal levels. Slightly higher levels of homocysteine in the region are due to multiple genetic and environmental factors.

Keywords**:** Type 2 Diabetes Mellitus, Homocysteine

Received: 16 April 2011
Accepted: 26 July 2011
Address communications to:Dr. Mohammad Javad Kajbaf, Razi Clinical Lab, Adham Avenue, No 406, Naderi Crossing, Ahwaz, Iran
Email:Dr.Mjkajbaf@yahoo.com

Introduction

Type 2 diabetes is the most common from of diabetes mellitus, representing about 90% of all diabetes cases worldwide (1). It is associated with a variety of complications including retinopathy, nephropathy, neuropathy, acute myocardial infarction, stroke and peripheral vascular disease. Microangiopathy seems to result from functional and metabolic disruption of the small vessels but macrovasucular events are more common (2). About 80% of patients die from thrombosis and 75% from cardiovascular events (3).

Homocysteine is a sulphur containing amino acid and an intermediate product in the metabolism of methionine. Hyperhomocysteinaemia is an important independent risk factor for atherosclerotic diseases in both diabetic and non diabetic subjects (4, 5). There are some controversies regarding association between diabetes and homocysteine. In a study on factors associated with serum homocysteine level in type 2 diabetic patients, it was shown that glomerular filtration rate, creatinine, triglyceride and fasting blood glucose were all directly associated with homocysteine level (6) while in another study mean fasting homocysteine level was significantly lower in type 2 diabetic patients than control subjects (7). Association between homocysteine and hypertension in diabetes has been discussed in a review article and it has been concluded that kidney failure leads to high homocysteine level and eventually causes cardiovascular diseases (8). Hyperhomocysteinaemia in diabetic patients may contribute to the development of chronic vascular complications (9).

In spite of many research works on homocysteine in diabetic subjects, the association between these two is not totally clear. The aim of this study was, therefore, to assess the serum homocysteine levels in uncomplicated type 2 diabetic patients and control subjects and comparing these with other investigations in the world.

Materials & Methods

This research was carried out in accordance with ethical principles laid down in the declaration of Helsinki, Finland in 1964. Eighty five diabetic patients with mean age of 57.65 years were selected on the basis of clinical and laboratory criteria (10) during 2010 in Ahwaz City, southwest of Iran. They had already been diagnosed as non-nephropathic by their physicians. Eighty five healthy subjects, age and sex matched, with mean age of 57.68 years were also chosen as controls.

Fasting blood samples were collected for all tests and fasting blood glucose (FBS), 2 hours postprandial glucose (2 hPP), triglyceride, total cholesterol and high density lipoprotein cholesterol (HDL-c) tests were performed by standard enzymatic and photometric technique (11) using RAXT biochemistry autoanalyzer (Dublin, Ireland). Commercially available kits made by Parsazmun Company (Karaj, Iran) were used and their instructions were followed. All these tests are based on the production of hydrogen peroxide in early steps and quinoneimine at the end. This end product is directly proportional to the amount of glucose, cholesterol and triglyceride in the specimen, respectively. Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and risk factor were then calculated.

Serum homocysteine level was measured by ELISA following the procedure of a kit supplied by Axis –Shield diagnostics Ltd., (Dundee, U.K.). Briefly, homocysteine coated microplate wells were subjected to the sera.

Anti homocysteine monoclonal antibody was later added. The next step consisted of adding enzyme conjugate containing antibody against the monoclonal antibody and peroxidase followed by addition of substrate and chromogen. The reaction was stopped by sulphuric acid and the absorbance was read at 450 nanometre.

Statistical analysis was performed by SPSS software for windows version 17(SPSS Inc; Chicago, Illinois, USA). T-test was used to compare groups mean statisticaslly. $P<0.05$ was considered significant.

Results:

In patients group, men were ranging from 37-88 years and women from 25-79 years. In control group, men were ranging from 36-85 years and women from 25-82 years. Demographic and metabolic parameters are shown in Table1. As can be seen, the mean age of patients and control subjects are very close with no significant difference. In choosing patients and controls, effort was made to differentiate them originally on the basis of blood sugar while blood lipid and lipoprotein levels were kept in the normal range. As is shown in Table1, only patients' LDL-c is slightly above the optimal range (<100mg/dl).

Table 1- Comparison of demographic and metabolic parameters of patients and controls

	Patients (n=85)	**Controls (n=85)**	***P* - Value**
Male Age y (n=37)	58.37 ± 11.39	58.40 ± 11.03	0.710
Female Age y (n=48)	57.10 ± 12.51	57.12 ± 12.68	0.873
Group Age y	57.65 ± 11.98	57.68 ± 11.94	1.000
Fasting blood sugar mg/dl	189.51 ± 66.85	90.61 ± 6.20	0.000
2 hPP* mg/dl	340.22 ± 109.67	116.25 ± 17.08	0.008
Triglyceride mg/dl	143.47 ± 34.89	111.67 ± 23.30	0.000
Total cholestrol mg/dl	178.95 ± 34.94	164.21 ± 23.15	0.000
HDL-c mg/dl**	44.86 ± 9.63	43.07 ± 8.88	0.333
LDL-c* mg/dl**	105.75 ± 33.68	91.31 ± 21.45	0.002
VLDL- c** mg/dl**	28.95 ± 7.35	23.26 ± 5.13	0.003
Group HCY*** μ mol/1**	13.64 ± 6.39	15.99 ± 7.33	0.364
Male HCY	15.52 ± 5.91	18.00 ± 8.04	0.250
Female HCY	12.19 ± 6.42	14.44 ± 6.34	0.739

Results are shown as mean ± standard deviations *P* - Value < 0.05 Significant

* 2 hours postprandial glucose

** High density lipoprotein cholesterol

*** Low density lipoprotein cholesterol

****=Very low density lipoprotein cholesterol

***** Homocysteine

In patients as a whole and with respect to the gender, homocysteine levels were generally lower compared to controls, but difference was not significant in any case.

Discussion

Comparing homocysteine levels in the present study with other reports showed that patients in other parts of the world usually had higher levels in comparison with controls, e.g. in India patients homocysteine was 16.5 μmol/l and in controls 11.4 (12), in Tunis, in patients 14.2 and in controls 11.6 (13), in Japan, male patients 12.2 against 10.7 in controls and female patients 9.9 against 8.4 in controls (6). By contrast, there are some reports, in agreement with our findings which show higher levels of homocysteine in controls compared to patients. For example in Italy patients homocysteine was 7.7 and in controls was 11.8 (7), also in Brazil in patients was 8.5 and in controls was 9.9 (14). Another study on young patients in USA showed almost the same level of homocysteine in patients and controls (8.1 against 7.3) so that the difference was not significant (15). Another study in Tunis also showed that the difference between patients and controls was not significant only in man (13). The factors responsible for higher levels of homocysteine in controls in the present study were not clear, although the difference was not significant. In fact, all individuals, either patient or control, have a low to moderate Level of homocysteine and the definition of elevated homocysteine levels is not standardized. It is, however, generally accepted that this is the severe hyperhomocysteinaemia which causes a wide range of diseases (16).

Homocysteine levels, ranging from 12.19 to 18.00, in both patients and controls as well as in males and females moieties (Table 1) were not high with respect to the age of population studied since the normal range up to 60 years is 5-15 (17). Normally, kidneys are responsible for more than 70% of homocysteine clearance. Therefore, reduced glomerular filtration rate with age and renal failure have been associated with increased serum homocysteine levels (18). In a recent study, serum homocysteine also showed to be an independent risk factor for the development of microalbuminuria in type 2 diabetic patients (19). In the present study, patients were not nephropathic and it is most likely that this is the main reason for showing normal levels.

Comparison of homocysteine levels of patients and controls with other reports shows somehow higher levels. Clearly, these variations are due to several factors like age, sex, size of population, individuals genetic make-up, nutritional regimens, etc. (20, 21).Therefore, all these factors should be kept homogenous to judge properly about these levels variations. Fortunately, today, there are both simple, inexpensive and non-toxic therapy with folic acid, vitamin B6 and B12 (16, 22) as well as treatment with lipopenic and hypotensive drugs (13) available to reduce the homocysteine levels in patients.

Conclusion

In patients as a whole and with respect to the gender, homocysteine levels were generally lower compared to controls. Homocysteine levels of patients and controls were slightly higher than other parts of the world, although the levels were in the normal range.

Acknowledgements

Authors thank Dr H Habili from Ahwaz Naft Grand Hospital, and M Songolzadeh from Razi Clinical Laboratory, Ahwaz, for helping in specimen collection, also, R Fouladvandi and B Norouzi, from Razi Clinical Laboratory

for technical assistance. Authors funded this work. There was no conflict of interest.

References

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414(6865):782-7.
2. Yamada T, Sato A, Nishimori T, Mitsuhashi T, Terao A, Sagai H, *et al.* Importance of hypercoagulability over hyperglycemia for vascular complication in type 2 diabetes. Diabetes Res Clin Pract 2000;49(1):23-31.
3. Carr ME. Diabetes mellitus: a hypercoagulable state. J Diabetes Complications 2001;15(1):44-54.
4. Glueck CJ, Shaw P, Lang JE, Tracy T, Sieve-Smith L, Wang Y. Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. Am J Cardiol 1995;75(2):132-6.
5. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346(8987):1395-8.
6. Masuda Y, Kubo A, Kokaze A, Yoshida M, Fukuhara N, Takashima Y. Factors associated with serum total homocysteine level in type 2 diabetes. Environ Health Prev Med 2008;13(3):148-55.
7. Mazza A, Bossone E, Mazza F, Distante A. Reduced serum homocysteine levels in type 2 diabetes. Nutr Metab Cardiovasc Dis 2005;15(2):118-24.
8. Sen U, Tyagi SC. Homocysteine and Hypertension in Diabetes: Does PPARgamma Have a Regulatory Role? PPAR Res 2010;Article ID:806538.
9. Rudy A, Kowalska I, Straczkowski M, Kinalska I. Homocysteine concentrations and vascular complications in patients with type 2 diabetes. Diabetes Metab 2005;31(2):112-7.
10. Definition, diagnosis and classification of diabetes mellitus and it's complications. 1999. Geneva, World Health Organization. Ref Type: Serial (Book,Monograph)
11. Burtis C, Ashwood E. Tietz textbook of Clinical Chemistry. 3 ed. Philadelphia: WB Saunders; 1999.
12. Narang AP, Verma I, Kaur S, Narang A, Gupta S, Avasthi G. Homocysteine--risk factor for ischemic stroke? Indian J Physiol Pharmacol 2009;53(1):34-8.
13. El OM, Aouni Z, Ouertani H, Mazigh C, Machghoul S. Effect of lipopenic and hypotensive treatment on homocysteine levels in type 2 diabetics. Vasc Health Risk Manag 2010;6:327-32.
14. Soares AL, Fernandes AP, Cardoso JE, Sousa MO, Lasmar MC, Novelli BA, *et al.* Plasma total homocysteine levels and methylenetetrahydrofolate reductase gene polymorphism in patients with type 2 diabetes mellitus. Pathophysiol Haemost Thromb;36(5):275-81.
15. Faulkner MS, Chao WH, Kamath SK, Quinn L, Fritschi C, Maggiore JA, *et al.* Total homocysteine, diet, and lipid profiles in type 1 and type 2 diabetic and nondiabetic adolescents. J Cardiovasc Nurs 2006;21(1):47-55.
16. Eikelboom JW, Lonn E, Genest J, Jr., Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131(5):363-75.
17. Frantzen F, Faaren AL, Alfheim I, Nordhei AK. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. Clin Chem 1998;44(2):311-6.
18. Mazza A, Giugliano D, Motti C, Cortese C, Andreotti F, Marra G, *et al.* Glycemia, MTHFR genotype and low homocysteine in uncomplicated type 2 diabetic patients. Atherosclerosis 2000;149(1):223-4.
19. Cho EH, Kim EH, Kim WG, Jeong EH, Koh EH, Lee WJ, *et al.* Homocysteine as a risk factor for development of microalbuminuria in type 2 diabetes. Korean Diabetes J 2010;34(3):200-6.
20. Schneede J, Refsum H, Ueland PM. Biological and environmental determinants of plasma homocysteine. Semin Thromb Hemost 2000;26(3):263-79.
21. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, *et al.* Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50(1):3-32.
22. Bazzano LA. Folic acid supplementation and cardiovascular disease: the state of the art. Am J Med Sci 2009;338(1):48-9.