

## Original Article

### Reduced Serum Homocysteine Levels in Diabetic Patients

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#### 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  $\mu\text{mol/l}$  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

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## Introduction

Type 2 diabetes is the most common form 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 macrovascular 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 statistically.  $P < 0.05$  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 Table 1. 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 Table 1, only patients' LDL-c is slightly above the optimal range ( $< 100 \text{ mg/dl}$ ).

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

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

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