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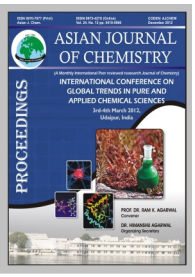

Homocystein Level in Patient with Ischemic Heart Disease and Some Associated Diseases†

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Homocysteine is an intermediate of methionine metabolism which, at elevated levels, is an independent risk factor for vascular disease and atherosclerosis. This study includes the determination of homocysteine level in random serum of patients with pure ischemia, ischemia with hypertension and ischemia with diabetes who attended Merjan teaching hospital in Hilla city, Babylon province, Iraq, from August, 2008 to May, 2009. Patients were divided into three groups; 23 patients had pure ischemic heart disease (17 males and 6 females), 16 had ischemic heart disease with hypertension (10 males and 6 females), 18 had ischemia heart disease with diabetes (8 males and 10 females). The control group consisted of 40 normal individuals who did not have any symptoms for ischemic heart disease and were gender and age-matched with the patients. Homocysteine level was measured by high performance liquid chromatography after deproteinized blood sample with 15 % 5 -sulphosalicylic acid. Homocysteine level was significantly increased ($P < 0.01$) in pure ischemic patients, ischemia with hypertension and ischemia with diabetes in comparison with control group. A comparison had also been done between male and female groups in patients and control groups and no significant changes ($P > 0.05$) were observed.

Key Words: Homocysteine, High performance liquid chromatography, Ischemic heart disease.

INTRODUCTION

Cardiovascular disease is a leading cause of death, accounting for one third of all deaths around the world, especially in industrialized countries. From all cardiovascular deaths, the majority is caused by ischemic heart disease (29 %) and strokes (23 %) alone, with heart failure adding another 14 %. Mortality from cardiovascular causes, however, is age-dependent: it rises from 17 % in people aged < 50 years to 37 % in the older age[1-4].

Hypertension, is referred to elevated blood pressure. Hypertension, is a medical condition in which the blood pressure is chronically elevated. In current usage, the word hypertension without a qualifier normally refers to systemic, arterial hypertension[5].

Hypertension can be classified into two types[6,7]: 1) Essential hypertension or primary hypertension, which indicates that no specific medical cause can be found to explain a patient's condition. About 90-95 % of hypertension is essential hypertension[7-9]. 2) Secondary hypertension which indicates that the high blood pressure is a result of *i.e.*, secondary to another condition, such as kidney disease or tumours (adrenal adenoma or pheochromocytoma)[9].

Diabetes mellitus is a syndrome of decreased carbohydrate, fat and protein metabolism caused either by lack of insulin secretion or decreased sensitivity of the tissues receptors to insulin. As a result of decreased insulin secretion or insulin resistance, blood glucose concentration increases, cells utilization of glucose fall increasingly lower and utilization of fats and proteins increase[8].

Homocysteine is an amino acid (formed by metabolism of methionin) with the chemical formula $HSCH_2CH_2CH(NH_2)CO_2H$ (Fig. 1).

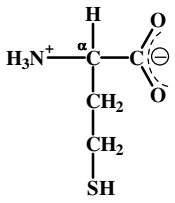

Fig. 1. Chemical structure of homocysteine

Homocysteine is an intermediate of methionine metabolism[10]. Ford *et al.*[11] and Allon *et al.*[10] noticed that elevated blood homocysteine concentration was an independent risk

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factor for cardiovascular disease. Willinek *et al.*[12] reported that high-normal serum homocysteine concentrations are associated with an increased prevalence of carotid artery wall thickening. The significance of the contribution of homocysteine to the variation of carotid intima-media thickness suggests a role for homocysteine as an independent risk factor for early carotid artery atherosclerosis in the asymptomatic subjects. Different studies indicated that the elevated level of total homocysteine (tHcy) had increased the risk of cardiovascular diseases and stroke[13]. Homocysteine is elevated in the case of inborn errors of methionine metabolism and excessive amount of homocysteine and its derivatives are found in blood and tissues of cardiovascular patients[14]. Moderate hyperhomocysteinemia up to (30 µmol/L) is a major independent risk factor of a number of diseases characteristic of old ages, primarily occlusive vascular disease[15].

EXPERIMENTAL

Ortho-phthalaldehyde reagent was prepared by dissolving 50 mg of *o*-phthalaldehyde in 1.25 mL of methanol and 50 µL of 2-mercaptoethanal was added and the volume was completed to 10 mL by addition of 0.4 molar sodium borate buffer. The pH was adjusted to 9.5 by addition of sodium hydroxide. The prepared sample was flushed with helium to expel dissolved oxygen and then stored in dark for use in 24 h time.

The stock solutions of amino acid were prepared in methanol with a concentration of 100 ppm. Standards mixture were prepared to obtain quantitative analysis by diluting the stock solution with methanol to obtain a final concentration of 10 ppm and then the mixture was divided into 1 mL portion and then was stored in -20 ºC.

Serum homocysteine levels were determined by using high performance liquid chromatography. Standard solution of homocysteine were used for comparison.

Blood was collected and sera were stored at -20 ºC until analysis. Homocysteine was measured by high-performance liquid chromatography at the Laboratories of Chemistry Department, Ministry of Science and Technology, Baghdad. Serum homocysteine concentration has been investigated using reversed phase chromatography. By reversed phase chromatography amino acids are separated after per column derivatization. Various reagents for per column derivatization of amino acids were used, such as naphthylisocynate and *o*-phthaladehyde[16,17].

The study was conducted during the period from Augest, 2008 to May, 2009 in Merjan hospital in Hilla city, Babylon province, Iraq. A total 57 patients were included from urban and rural area (35 males and 22 females). Twenty three patients had pure ischemic heart disease (17 males and 6 females), 18 had ischemic heart disease with hypertension (10 males and 6 females), 18 had ischemia heart disease with diabetes (8 males and 10 females). The age of the patients varying between 40-85 years old (mean ± SD 57.82 ± 10.25).

The control group consisted of 40 healthy person who were chosen as healthy, non smokers, didn't have any history of chronic disease and didn't take any treatment for chronic diseases, as diabetes mellitus and hypertension. Full history, physical examination and investigations which included blood sugar to exclude diabetes and electrocardiography to look for any evidence of ischemia were done. The age of control group was varying between 40-85 year (mean ± SD 58 ± 7.5) from urban and rural area (20 males and 20 females).

Sample preparation: 200 µL of frozen serum sample after complete thawing was deproteinized by 25 µL of 15 % 5-sulphosalicylic acid, mixed and centrifuged at 8000 rpm for 10 min time.

Retention time of homocysteine: Retention time of standard solutions of homocysteine and serum samples was found to be in between 2.948 and 2.988 min (Fig. 2a and b). Homocysteine level was obtained by comparison with standard solution at the same conditions of HPLC for beaks at the same retention.

Separation of homocysteine on reversed phase column C_{18} (250 × 4.6 mm c.d), mobile phase 0.001 M buffer phosphate: methanol 70 :30 (v/v), flow rate = 0.5 mL/min in room tempreture, detection at 265 nm[18,19].

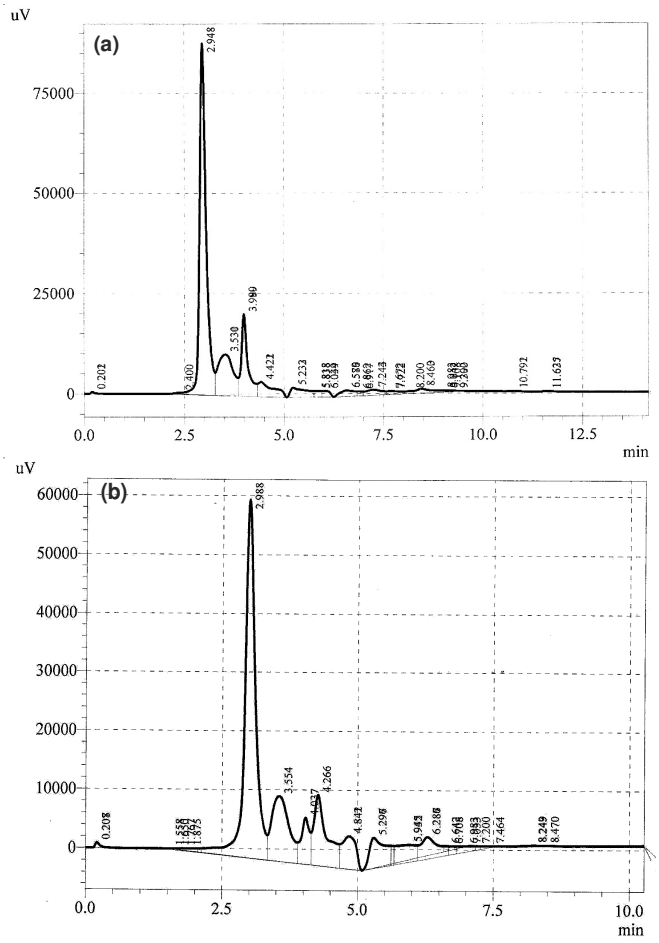

Fig. 2. HPLC chromatogram for homocysteine (a) standard (b) serum sample

RESULTS AND DISCUSSION

The patients and control were divided according to ages into two groups group 1 in which the age range between 40-60 year and age group two in which the age is > 60 year as shows in Table-1.

The sex distribution of total ischemic patients, was clearly obvious. The highest percentage in male was 61 % (35 out of 57 patients) while female was 39 % (22 out of 57 patients) (Fig. 3).

TABLE-1
PATIENTS AND CONTROL NUMBER ACCORDING TO AGE GROUP

Patients		Age	
		40-60 year	60 year >
Ischemic patients	Pure ischemia	11	12
	Ischemia with hypertension	4	12
	Ischemia with diabetes	8	10
Control		24	16

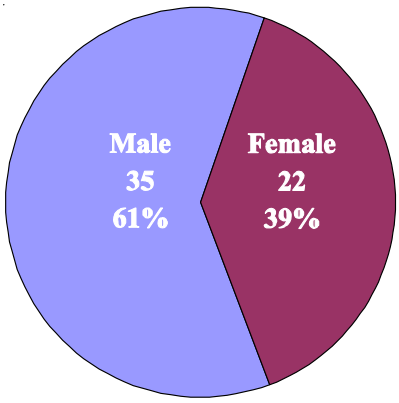

Fig. 3. Sex distribution of total ischemic patients

Table-2 shows the sex distribution for all patients in this study. Number of female patients in ischemia with diabetes were more than male patients while in ischemia and ischemia with hypertension male patients were more than female.

TABLE-2
SEX DISTRIBUTION IN THE STUDY GROUPS

	Groups	Number			
		Male	%	Female	%
Patients	Pure ischemic patients	17	31	6	14
	Ischemic patients with hypertension	10	18	6	14
	Ischemic patients with diabetes	8	15	10	24
	Control	20	36	20	48
	Total	55		42	

Measurements of homocystein in 40 control groups (20 male and 20 female aging between 40-85 years show no significant changes ($p > 0.05$) between male and female. Table-3 shows homocystein concentration (μmol/L) in control group that measured by HPLC.

TABLE-3
HOMOCYSTEIN LEVEL (μmol/L) IN CONTROL GROUP

Number of subjects	Sex group (years)	Concentration range	Mean ± SD
40	(m+f)	8.12-11.20	19.6 ± 1.58
20	(m)	8.35-11.20	19.92 ± 0.85
20	(f)	8.12-10.51	19.26 ± 0.85

$P > 0.05$

Measurements of homocystein in two age groups (40-60) and > 60 years show no significant changes in their levels ($p > 0.05$). Table-4 shows comparison of homocystein concentration in control group between two age groups.

Comparison between ischemic male or female and similar sex healthy controls results indicate an increase in level of homocysteine among patients groups. This elevation in homocysteine concentration value was highly significant ($P < 0.01$). When similar comparisons were done between ischemia with hypertension and ischemia with diabetes and control group a similar behaviour were observed ($P < 0.01$). Table-5 shows the mean values of serum level of homocysteine in male and female compared with the mean values in control group. No significant changes was observed when comparison was done between two age groups as shown in Table-6.

TABLE-4
COMPARISON OF HOMOCYSTEIN CONCENTRATION IN CONTROL GROUP BETWEEN TWO AGE GROUPS

Age group	Number	Mean ± SD (μmol/L)
40 -60	24	9.41 ± 0.92
> 60	16	9.26 ± 0.68

$P > 0.05$

TABLE-5
MEAN SERUM LEVEL OF HOMOCYSTEINE IN MALE AND FEMALE PATIENTS GROUPS AND CONTROL GROUP

Group	Male	Female
	Mean ± SD	Mean ± SD SDSD
Control	9.58 ± 0.93	9.11 ± 0.65
Ischemic patients	24.11** ± 5.45	25.4** ± 2.5
Ischemia with hypertension	20.84** ± 3.38	23.9** ± 4.85
Ischemia with diabetic	22.16** ± 3.59	21.26** ± 1.44

** $P < 0.01$

TABLE-6
COMPARISON OF LEVELS OF HOMOCYSTEINE (μmol/L) BETWEEN TWO AGE GROUPS [AGE GROUP 1 (40-60) AND AGE GROUP 2 (>60)]

Age group	Pure ischemia	Ischemia with hypertension	Ischemia with diabetes
40-60	23.29 ± 5.3	19.83 ± 1.81	24 ± 4.62
> 60	25.32 ± 4.93	22.88 ± 4.87	21.02 ± 1.23

There is a highly significant increase in homocysteine level in ischemic patients compared with control values ($P < 0.01$). Also there is significant increase in homocysteine level in patients with ischemia with hypertension and ischemia with diabetes compared with control patients as shown in Table-7.

TABLE-7
MEAN SERUM LEVEL OF HOMOCYSTEINE (μmol/L) IN DIFFERENT PATIENTS GROUPS AND CONTROL GROUPS

Groups	Mean (μg/mL ± SD)
Control	9.35 ± 0.82
Ischemic patients	24.31 ** ± 5.09
Ischemia with hypertension	22.46** ± 4.61
Ischemia with diabetic	21.84** ± 2.96

**$P < 0.01$

There is no significant changes in serum homocysteine level between pure ischemic patients, ischemia with hypertension and ischemia with diabetes ($P > 0.05$).

The relationship between increased homocysteine and heart disease is well established in the medical community. Unlike the other three predictors of heart disease, which are cholesterol, triglycerides and C reactive protein, homocysteine levels are influenced by what the person does not eat rather than what he does eat. This is due to the fact that homocysteine

is a sulphydryl-containing amino acid derived from demethylation of methionine. Nutritional deficiencies in the vitamin cofactors (folate, vitamin B12 and vitamin B6) required for homocysteine metabolism may promote hyperhomocysteinaemia[20]. This means that increased homocysteine levels are associated with increased risk of cardiovascular disease and then tHcy measurement will become another useful marker of vascular risk, multivitamin therapy will be another therapeutic option for people at risk of atherothrombotic vascular disease and fortification of food with folic acid will rise high on the political and public health agenda. Homocysteine level in all patients with heart disease in this study (pure ischemia, ischemia with hypertension and ischemia with diabetes) were found to be elevated significantly ($P < 0.01$) compared with control groups. Only about two-thirds of all episodes of symptomatic atherothrombotic vascular disease in developed countries can be attributed to established genetic and environmental vascular risk factors[21]. An additional causal vascular risk factor may be raised plasma levels of homocysteine (hyperhomocysteinaemia).

Mild hyperhomocysteinaemia occurs in approximately 6 % of the general population[22,23].

Patients with mild hyperhomocysteinaemia are typically asymptomatic until the third or fourth decade of life when premature coronary artery disease develops, as well as recurrent arterial and venous thrombosis. The elevation of serum level of homocysteine (hyperhomocysteinemia) in this study could be considered as a risk factor for cardiovascular disease. Research has shown that increased homocysteine level is associated with both the hyperinsulinemia seen with insulin resistance and increased urinary albumin excretion. It is also associated with low serum levels of vitamin B12[24]. However, supplementation with vitamin B12 has resulted in reduction of homocysteine levels, but as failed to show subsequent reductions in incidence of cardiovascular disease[25].

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