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Homocysteine, an Early Predictor of Cardiovascular Risk in Type 2 Diabetes Mellitus

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Abstract
Introduction: The amino acid, homocysteine has been a "topic of interest" in vascular disease in recent years. There are indications that increased homocysteine concentrations can lead to cardiovascular disease and mortality in general population. Though high concentration of plasma homocysteine is seen in patients with diabetes mellitus, its relationship with cardiovascular morbidity is not yet established.
Objective: This was a prospective study conducted to evaluate serum homocysteine concentration as an early marker for diagnosis of atherosclerosis in patients with type 2 diabetes mellitus.
Materials and Methods: The study enrolled 60 patients including both male and female with an average age range from 30 to 75 years. The study patients were divided into three groups: Group I (Control group): Twenty healthy volunteer controls were selected to be about the same age of the patients; Group II (Uncomplicated diabetic group): Twenty patients with long history of type 2 diabetes without any accompanying diseases especially, coronary heart diseases and Group III (Complicated diabetic group): Twenty patients with long history of type 2 diabetes together with coronary heart diseases. Glycosylated hemoglobin was estimated by chromatographic-spetrophotometric ion exchange method, homocysteine was measured by ARCHITECT Homocysteine Reagent Kit method, blood glucose was measured glucose oxidase method and cholesterol was measured by enzymatic method.
Results: A significant difference was seen in serum homocysteine ($p<0.001$) between group I and group III. A significant difference in the serum total cholesterol ($p<0.001$) between group I and group III was observed. For serum triacylglycerol test, a significant difference ($p<0.001$) was noted between group I and group II, and between group I and group III. There was a significant difference in serum lipids risk ratio ($p<0.001$) between group I and group III. The mean ± SD of serum urea (mg/dl) in group I was 33.10±6.21, in group II 41.05±11.29 and in group III 37.20±7.36. A significant difference ($p<0.001$) was seen between group I and group II, and between group I and group III. A significant microalbuminuria ($p<0.001$) was seen between group I and group II, and between group I and group III. There was a significant increase in the correlation between serum homocysteine and lipid risk ratio of group.
Conclusion: The study concluded that serum homocysteine determination could be used as a sensitive, early and more accurate tool for screening and monitoring early detection of atherosclerosis.

Keywords: Homocysteine, Type 2 diabetes mellitus, cardiovascular disease

1. Introduction
It has been suggested that diabetes have a two to four-fold greater risk of vascular disease occurrence as compared with non-diabetes, and atherosclerosis develops even in the presence of mild impairment of glucose tolerance [1]. The sulfhydryl-containing amino acid, homocysteine has been a "topic of interest" in vascular disease in recent years. In addition to arteriosclerosis and heart disease, the importance of homocysteine has also rapidly expanded to areas of biology, physiology, and medicine ranging from endothelial function to aging, oxidative stress, oxidative metabolism, embryology, reproductive physiology, cancer, growth and cell division, endocrinology, neural transmission, and neurodegenerative disease [2]. There are indications that increased homocysteine concentrations can lead to cardiovascular disease and mortality in general population [3].
Though high concentration of plasma homocysteine is seen in patients with diabetes mellitus, its relationship with cardiovascular morbidity is not yet established. Therefore, there is a need of conducting large prospective studies to follow the effects of homocysteine and its normalization on accelerated atherosclerosis in diabetes [4]. The aim of the current prospective study was to evaluate serum homocysteine concentration as an early marker for diagnosis of atherosclerosis in patients with type 2 diabetes mellitus.

2. Materials and Methods

The study enrolled 60 patients including both male and female with an average age range from 30 to 75 years. The patients were selected from the outpatient department of Diabetes Mellitus Clinic, Seventeenth of February Teaching Hospital, Al- Baida. Laboratory work-up was carried out at Biochemistry Department, Omar Al-Mukhtar Faculty of Medicine and Clinical Laboratory at Seventeenth of February Teaching Hospital, Al-Baida. Homocysteine was investigated at Al-Mokhtbar Laboratory, at Cairo, Egypt. The study patients were divided into three groups:

- Group I (Control group): Twenty healthy volunteer controls were selected to be about the same age of the patients.
- Group II (Uncomplicated diabetic group): Twenty patients with long history of type 2 diabetes without any accompanying diseases especially, coronary heart diseases. All patients of this group had history of diabetes for at least 5 years.
- Group III (Complicated diabetic group): Twenty patients with long history of type 2 diabetes together with coronary heart diseases.

The patients underwent following biochemical examinations:

2.1 Blood and Urine Samples

A total of 8 milliliter (ml) blood was left to fill the syringe with minimal pistol withdrawn after removal of tourniquet. The blood was examined for the following tests:

- Two ml of the blood was added to potassium EDTA as an anticoagulant for determination hemoglobin A1C (glycosylated hemoglobin) using chromatography-spectro-photometric ion exchange method [5].
- Two ml of the blood was added to fluoride oxalate as an anticoagulant for determination of fasting plasma glucose.
- Four ml blood was collected and left at room temperature for 20 minutes to clot. The serum was separated by centrifugation at 3000 r. p.m. for 10 minutes, and kept in (-20 °C) till analysis for homocysteine, urea, creatinine and lipid profile.
- Random urine samples were collected to test microalbuminuria by enzyme-linked immunosorbent assay (ELISA) technique [6].

2.2 Homocysteine Assay

Homocystine was measured by ARCHITECT Homocysteine assay, an immunoassay for the quantitative determination of total L-homocysteine in human serum or plasma. It uses a 4 Parameter logistic Curve Fit (4PlC, Y-weighted) data reduction method to generate a calibration curve [7].

3. Results

The results of serum homocysteine (mean± SD) among the study groups control (group I), group II and group III are shown in Table 1. There is a significant difference in serum homocysteine ($p<0.001$) between group I and group III. There was no significant difference of serum homocysteine ($p>0.05$) between group I and group II, and between group II and group III. The results of the diabetic profile (mean±SD) among the studied groups control (group I), group II and group III are depicted in Fig. 1. A significant difference was seen in fasting plasma glucose ($p<0.001$), and in hemoglobin A1c ($p<0.001$), when comparing control (group I) with two studied groups in two parameters.

Table 1: Serum Homocysteine (umol/L) in control and two other studied groups (number = 20 each).

	Control (group I)	Group II (group I vs. Group II)	Group III (group I vs. Group III)	(Group II vs. Group III)
Range	6.35 – 15.73	7.4 – 47.34	10.17 – 21.51	
Mean	12.49	14.67	15.52	
S.D.	± 2.02	± 8.917	± 3.29	
t-test		- 1.068	-3.056	-0.400
P-value		0.293	< 0.001	0.691

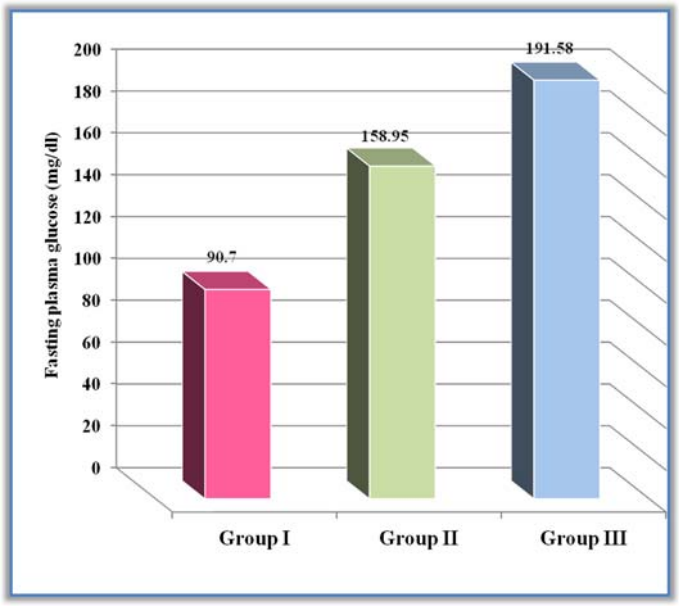

Fig 1: Mean levels of Fasting plasma glucose (mg/dl) in the study groups.

3.1 Lipid Profile Data of Study Patients

The results of lipid profile tests (mean ± SD) among the study groups, control (group I), group II and group III are presented in Table 2. A significant difference in the serum total cholesterol ($p<0.001$) between group I and group III was seen. For serum LDL cholesterol, a significant difference ($p<0.05$) was seen between group I and group III and between group II and group III, while there is no significance difference of serum LDL cholesterol ($p>0.05$) between group I and group II. No significant difference was observed in all the study groups for serum HDL cholesterol. For serum triacylglycerol test, a significant difference ($p<0.001$) was noted between group I and group II, and between group I and group III. There was no significant difference ($p>0.05$) between group II and III. There was a significant difference in serum lipids risk ratio ($p<0.001$) between group I and group III, and between group II and group III. Whereas, no significant difference ($p>0.05$) was seen between group I and group II.

Table 2: Lipid profile data of study groups

		Control (Group I)	Group II (Group I vs. Group II)	Group III (Group I vs. Group III)	(Group II vs. Group III)
Serum total cholesterol (mg/dl)	Range	74 – 178	70 – 289	97 – 251	
	Mean	124.65	150.2	171.2	
	S.D.	± 29.16	± 52.62	± 47.246	
	t-test		-1.825	- 3.733	-1.33
	P- value		0.084	< 0.001	0.192
Serum LDL cholesterol (mg/dl)	Range	35 – 126	20 – 187	28 – 177	
	Mean	70.70	67.90	106.30	
	S.D.	± 29.68	± 42.18	± 39.45	
	t-test		0.223	-3.938	-2.674
	P- value		0.826	< 0.001	< 0.001
Serum HDL cholesterol (mg/dl)	Range	18 – 86	21 – 50	18 – 39	
	Mean	46.15	35.65	28.10	
	S.D.	± 18.58	± 8.66	± 5.37	
	t-test		2.44	4.129	3.978
	P- value		< 0.001	< 0.001	< 0.001
Serum triacylglycerols (mg/dl)	Range	87 – 233	65 – 347	76 - 243	
	Mean	136.60	163.6	162.7	
	S.D.	± 39.83	± 82.25	± 59.54	
	t-test		-1.162	-3.291	0.036
	P- value		< 0.001	< 0.001	0.971
Serum lipids risk ratio	Range	1.2– 9.8	1.6 – 10.3	2.8 – 10.3	
	Mean	3.17	4.5	6.255	
	S.D.	± 1.905	± 2.275	± 2.072	
	t-test		-2.005	-4.902	-2.551
	P- value		0.052	0.0001	0.015

The mean ± SD of serum urea (mg/dl) in group I was 33.10±6.21, in group II 41.05±11.29 and in group III 37.20±7.36. A significant difference (p<0.001) was seen between group I and group II, and between group I and group III. A significant microalbuminuria (p<0.001) was seen between group I and group II, and between group I and group III. There was a significant increase in the correlation between serum homocysteine and lipid risk ratio of group (see Fig. 2).

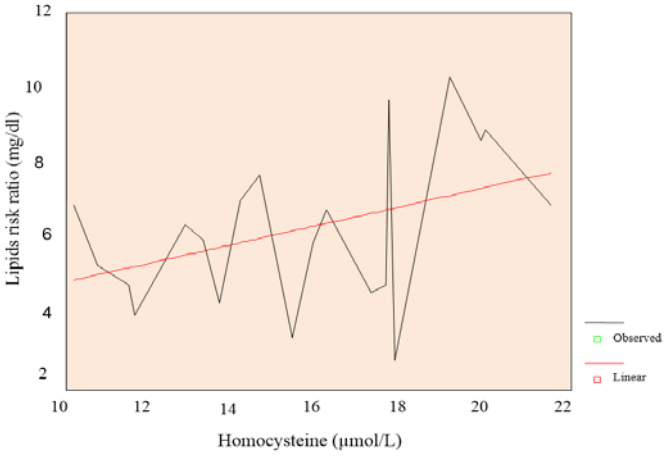

Fig 2: Correlation between serum homocysteine and lipid risk ratio of group III.

4. Discussion

Homocysteine is a non-protein amino acid, which can be biosynthesized from methionine by the removal of its terminal C methyl group. While detection of high levels of homocysteine has been linked to cardiovascular disease, lowering homocysteine levels may not improve outcomes [8]. Epidemiological studies have established hyperhomocysteinemia as an independent risk factor for cardiovascular disease, cerebrovascular disease, dementia-type disorders, and osteoporosis-associated fractures. Although combined folic acid and B-vitamin therapy substantially reduces homocysteine levels, results from randomized placebo-controlled clinical trials testing the effect of vitamin therapy on outcome in these diseases are mixed, but have generally fallen short of expectations [9]. In type 2 diabetes mellitus, homocysteine level is elevated and may be stronger risk factor for cardiovascular diseases than non-diabetic subjects [10].

The results of the current study depicted a higher increase in serum homocysteine concentration in diabetic patients (group II and III) when compared to control group. Such increase is statistically high in complicated diabetic group III when compared to control group. This indicates that as the diabetes disease got advanced and complicated with some cardiovascular diseases, homocysteine concentration got higher. In this study the homocysteine results are in agreement with the results of Hoogeveen *et al.*, 1998, [10] Hultberg *et al.*, 1997 [11] and Bots *et al.*, 1998, [12] who stated that homocysteine is markedly elevated type 2 diabetes mellitus patients with inadequate glycemic control.

The mechanism by which homocysteine may increase the risk of vascular disease has not yet been clearly established. However, in one of the study it was stated that hyperhomocysteinemia stimulates vascular smooth muscle cell growth, leading to intimal arterial wall thickening which may reduce oxygen transmissibility, within the wall and give rise to oxygen free radicals with subsequent tissue damage [13]. Similarly, the reduction of molecular oxygen coupled to the oxidation of homocysteine may result in free radicals and hydrogen peroxide, which may damage endothelial cells [14]. Hyperhomocysteinemia, possibly by increasing oxidant stress as well as by other mechanisms, may induce dysfunction of the vascular endothelium and proliferation of vascular smooth muscle cells, both key processes in atherogenesis [15].

The risk of thromboembolic vascular diseases increases with increased levels of serum homocysteine. Possible mechanism for the thrombogenic states associated with hyperhomocysteinemia include effects on endothelial cells,

platelets, clotting factors or disorders in interactions of any of these factors. Homocysteine could alter the surface of endothelial wall changing its phenotype from anticoagulant to procoagulant. Moreover, it partially inhibits collagen cross-linking and this lathyritic collagen is very effective in platelets aggregation in areas enriched in collagen [16]. The present study revealed statistically significant increase in the cholesterol levels in group III complicated diabetic patients when compared to control group. Such significance was absent between control group and uncomplicated diabetic group II and between uncomplicated and complicated diabetic groups II and III. Significant high concentration of triacylglycerols was found in uncomplicated and complicated groups II and III when compared to control group. These lipid profile results are in agreement with the results of a study conducted by Watkins *et al.*, 1990 [17].

The present study revealed statistically insignificant correlations between lipid risk factors and homocysteine concentration in study groups as indicated by correlation coefficient studies. The study results showed that kidney function tests (urea and creatinine) concentration in diabetic patients (group II and III) were higher than in normal controls (group I). As the diabetes disease got advanced and complicated, these kidney functions got worse. This was indicated by statistically highly significant increase in the creatinine levels in complicated diabetic patients (group III) when compared to uncomplicated diabetic group II. Our study showed that the incidence of microalbuminuria in diabetic patients was statistically higher than in normal controls. Such difference was absent in complicated diabetic patients when compared to uncomplicated diabetic group. At the same time, it was also noted that statistically insignificant positive correlations between microalbuminuria and homocysteine concentration in the diabetic groups as indicated by correlation coefficient studies. These results are in agreement with Parving *et al.*, 1992, who reported that the prevalence of microalbunuria was significantly higher in type 2 diabetes than non-diabetic patients [18].

5. Conclusion

Type 2 diabetes mellitus continues to be a serious health concern in the growing world. Cardiovascular complications, especially atherosclerosis in type 2 diabetes mellitus patients are an important event to be attended at the earliest. Studies have shown that high homocysteine concentration is prevalent in diabetic patients, and detection of high levels of homocysteine has been linked to cardiovascular diseases. The current study concluded that serum homocysteine determination could be used as a sensitive, early and more accurate tool for screening and monitoring early detection of atherosclerosis.

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