Turkish Journal of Endocrinology and Metabolism, (2005) 1 : 5-8 **ORIGINAL ARTICLE**

Levels of Plasma Homocysteine in Subjects with Impaired Fasting Glucose

Erkan Çoban* **Gökhan Yazıcıoğlu**** **Ramazan Sarı****

** Akdeniz University Faculty of Medicine, Department of Internal Medicine, Antalya, Turkey*
*** Akdeniz University Faculty of Medicine, Division of Endocrinology and Metabolism, Antalya, Turkey*

Impaired fasting glucose (IFG) is probably a frequent glycemic disorder in the general population and is considered as a prediabetic state. Hyperhomocysteinemia is an independent cardiovascular risk factor. The present study was designed to evaluate homocysteine levels in subjects with IFG compared with normal subjects and those with diabetes mellitus. Age, sex and body mass index matched 40 normoglycemic healthy subjects, 40 subjects with IFG (fasting glucose 110 to 125 mg/dl), and 40 patients with type 2 diabetes (fasting glucose ≥ 126 mg/dl) were included in the study. The levels of plasma homocysteine in patients with type 2 diabetes mellitus, IFG, and normal subjects were 14.8±2.7 µmol/, 12.4±2.1 µmol/, and 11.1±1.4 µmol/l, respectively. Subjects with IFG had significantly lower homocysteine levels than type 2 diabetic patients (p<0.000). There were significantly higher homocysteine levels in subjects with IFG than in normal subjects (p=0.018). Our data suggest that one possible mechanism by which subjects with IFG may be at increased cardiovascular risk.

Keywords: Impaired fasting glucose, diabetes mellitus, homocysteine, cardiovascular risk

Introduction

In 1997, the American Diabetes Association (ADA) proposed new criteria for defining diabetes based on fasting plasma glucose. A new diagnostic entity, impaired fasting glucose (IFG). In subjects with IFG, fasting plasma glucose concentrations range between 110 and 125 mg/dl. IFG is probably a frequent glycemic disorder in the general population and is considered as a prediabetic state (1). IFG is also associated with cardiovascular disease, but it is unclear whether it is an independent risk factor because it commonly coexists with other cardiovascular risk factors present in the metabolic syndrome (2,3).

Homocysteine (Hcy) is a metabolic product of methyl group donation by the amino acid methionine (4). Elevated plasma Hcy levels are contributing to development of atherosclerosis independent of standard cardiovascular disease risk factors in diabetic and non diabetic subjects (5). On the other hand, levels of plasma Hcy are frequently elevated in patients with coronary, cerebral and peripheral arterial occlusive diseases (6-8).

Several studies have shown that DM is associated with increased levels of Hcy (9-11). However, there has been no study about levels of Hcy in subjects with IFG. Therefore, the present study was designed to evaluate plasma Hcy levels in subjects with IFG compared with normal subjects and those with type 2 diabetes.

Patients and Methods

Patients

We selected 40 normoglycemic healthy subjects, 40 subjects with IFG (fasting glucose 110 to 126 mg/dl), and 40 newly diagnosed uncomplicated type 2 diabetic patients (fasting glucose ≥ 126 mg/dl) matched for age, gender, body mass index and occupation. Women were also matched for menopausal status. Patients with history of smoking, sustained hypertension, hyperlipidemia,

Correspondence address:

Erkan Çoban
Akdeniz Üniversitesi, Tıp Fakültesi İç Hastalıkları Anabilim Dalı
Arapsuyu, 07070 Antalya
E-mail : ecoban@akdeniz.edu.tr

obesity (BMI ≥ 30 kg/m²), cerebrovascular disease, ischemic heart disease, congestive heart failure, malignancy, renal failure (serum creatinine > 1.5 mg/dl, blood urea nitrogen > 30 mg/dl), hepatitis, alcoholism, high serum uric acid, psoriasis, confirmed macrocytic anemia and patients who were on medications like nitrate and vitamine preparations were excluded from the study. Study population had a similar dietary pattern. Smokers and non-smokers were grouped as their current smoking status. The BMI was calculated as the weight (kg)/height squared (m)². Informed consent was obtained from all patients. We measured levels of plasma Hcy in all groups.

Measurement of plasma total homocysteine

The plasma specimens were drawn after a fasting period of 12 h and were kept in tubes containing EDTA. Plasma got seperated from the red blood cells within 1 h of collection as synthesis and excretion of Hcy continued in the cells after sampling. Prior to analysis all specimens were stored in a frozen state (–20°C). We applied two levels of internal quality standards before assay. Level 1 had a value in the normal range and level 2 had a value above the threshold.

The disulphide bands, in the calibrant/sample were reduced using the reducing agent. Protein was precipitated from solution and the thiol groups in the supernatant were than derivatised with a fluorescent thiol-spesific dye. The fluorescent derivative mixture was then seperated using the Drew DS 30 Hcy analyser which automatically calculates the Hcy concentration using suitable derivates which are seperated and detected by their fluorescence (λex=385 nm, λem=515 nm). Quantitative evaluation of the Hcy concentration was achieved by comparison with a two-point calibration.

Statistical analysis

Statistical analysis was done by SSPS 10.0 statistical software. Data are expressed as mean ± SD. Groups were compared with one-way ANOVA followed by Bonferroni test. A p value of <0.05 was considered statistically significant.

Results

The main characteristics of study population are reported in Table 1. Age, gender distribution, and BMI did not differ among the groups by selection. Metabolic parameters were not different among the study groups as a result of the selection process.

The plasma levels of Hcy in patients with type 2 diabetes mellitus, IFG, and normal subjects were 14.8±2.7 μmol/, 12.4±2.1 μmol/, and 11.1±1.4 μmol/l, respectively. Subjects with IFG had significantly lower Hcy levels than type 2 diabetic patients (p<0.000). There were significantly higher Hcy levels in subjects with IFG than in normal subjects (p=0.018) (Table 1). Other laboratory parameters were not different among the study groups (data not shown).

Table 1. Clinical characteristics, fasting glucose and plasma homocysteine levels.

Parameters	Diabetic group	IFG group	Normal group
No	40	40	40
Sex (males/females)	21/19	20/20	20/20
Age (years)	47.22±9.23	48.11±9.46	47.28±9.56
BMI (kg/m²)	23.6±4.59	23.5±5.2	23.4± 5.1
Fasting glucose (mg/dl)	144 .23±13.82**†	113.28±11.84*	85.48±10.34
Homocysteine (μmol/l)	14.8±2.7**†	12.4±2.1*	11.1±1.4

IFG, impaired fasting glucose; BMI, body mass index
* p<0.05 vs. normal group, ** p<0.000 vs. normal group,
† p<0.000 vs. IFG group.

Discussion

IFG is probably a frequent glycemic disorder in the general population and is considered as a prediabetic state (1). However, cardiovascular risk associated with IFG has been examined various studies with conflicting results (12-18). Balkau et al. found a linear relationship between death from cardiovascular disease and fasting blood glucose levels (13). Bjørnholt et al. described the excess risk of cardiovascular deaths in nondiabetic men in the upper normal range of fasting blood glucose (19). In the Rancho Bernardo Study, an increase of fasting glucose from 5 to 7 mmol/l was associated with a doubling of cardiovascular disease mortality in men a tripling in women (12). On the contrary, Tominaga et al. concluded that impaired glucose tolerance was a risk factor for cardiovascular disease but not IFG (15). Crook et al. reported that serum total sialic acid, a strong cardiovascular risk factor with increased concentrations being

associated with increased mortality, is not elevated in subjects with IFG (18).

Recently Duncan et al. reported that fasting Hcy was higher subjects with IFG, compared to normal fasting glucose, and diabetic subjects had levels similar to non-diabetic subjects with IFG (20). In this selected population, we have found lower Hcy levels in subjects with IFG in comparison with type 2 diabetic patients. There were significantly higher Hcy levels in subjects with IFG than in normal subjects.

Some mechanisms have been proposed to explain the increased vascular risk induced by higher plasma Hcy levels. These include increased oxidant stress, impaired endothelial function, stimulation of mitogenesis, and induction of thrombosis; by impairing nitric oxide-dependent vasodilatation; and by enhancing oxidation of LDL cholesterol (5,21). In this context, Vehkavaara et al. have recently shown endothelial dysfunction in subjects with IFG (22). Also, elevation of plasma Hcy levels, possibly in relation to insulin resistance, may contribute to cardiovascular risk in relatives of subjects with IFG (23-25).

The present study has some limitations. First, since we excluded patients with clinically overt cardiovascular disease (such as coronary artery disease, cerebrovascular disease and renal failure) were excluded to clarify the spesific levels of glucose-related abnormalities. For this reason, our results cannot be extrapolated to all subjects with IFG. Second, since we did not evaluate genotypes of enzymes involved in Hcy metabolism and folic acid, vitamin B6 and vitamin B12 status, it is unclear whether the present results are related to genetic or nutritional factors. However, regardless of the mechanism, higher Hcy levels represent a risk factor for vascular disease. Thus, this limitation does not lessen the clinical relevance of our results.

In conclusion, our data suggest that one possible mechanism by which subjects with IFG may be at increased cardiovascular risk.

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