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Original Article

Variation in the visfatin gene may alter the required dosage of Oral antidiabetic agents in type 2 diabetic patients

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Abstract

Background: Treatment of diabetes with oral antidiabetic agents is accompanied by considerable variability in pharmacokinetics and clinical efficacy. Genetic factors may contribute to individual differences in bioavailability, drug transport, metabolism and drug action. We investigated the role of visfatin gene polymorphism (rs2110385) on required dosage of oral antidiabetic agents in type2 diabetic patient.
Methods*:* As a cross-sectional study, we recruited 94 patients with type 2 diabetes. Laboratory measurements were FBS, OGTT, HbA1C, fasting serum visfatin and Insulin. HOMA-IR and QUICKI indices were calculated. Genotyping for SNP was performed using the PCR-RFLP method. We recorded the amount of antidiabetic agents in the last 8 weeks before the survey according to drug dose (metformin 500mg and glibenclamide 5 mg).
Results: We found no significant difference in FBS, G2h, HbA1C levels, Fasting insulin concentration, and HOMA and QUICKI indices between various genotypes. The required dose of glibenclamide for adjustment of glucose homeostasis was lower in genotype GG compared to others, but there was no difference in required dose of metformin between various genotypes.
Conclusion: It seems that visfatin gene variation modifies the insulin secretion by glibenclamide treatment.

Keywords: Visfatin, Genotype, Antidiabetic agents, HOMA, QUICKI, Type 2 diabetes

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Introduction

Type 2 diabetes mellitus (T2DM) is associated with significant morbidity, including an increased risk of cardiovascular diseases and stroke, hypertension, retinopathy and blindness, end-stage renal disease, and neuropathy leading to amputations (1-3). The prevalence of diabetes mellitus has been rapidly increasing, stimulated by an tremendous increase in obesity and other metabolic risk factors, recently (4-6). Increased central adipose tissue is a common metabolic feature of type2 diabetes (7). The release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic subinflammatory state that could play a central role in the development of insulin resistance and type 2 diabetes (8-11).

Visfatin is recently described as an adipokine, previously recognized as a pre-B cell colony-enhancing factor (PBEF), comes into focus to play an important role in regulation of glycemic homoeostasis (12). Also, recent studies suggest that plasma visfatin concentration is increased in individuals with abdominal obesity and/or type 2 diabetes mellitus (13). Other studies revealed that circulating visfatin concentr-ation is affected by blood glucose levels; accordingly, may be affected with using some drugs (14).

A recently performed study demonstrated that intensive glycemic control lowered plasma visfatin levels in patients with type 2 diabetes and it suggested that the changes of visfatin concentration may be a compensatory mechanism to ameliorate insulin deficiency due to pancreatic beta-cell failure (15).

It has been reported that there are single nucleotide polymorphisms (SNPs) in promoter region of visfatin gene that accompanied with susceptibility to T2DM, and some of them, have been demonstrated to significantly correlate with glucose homeostasis (16). Polymorphism studies on the coding regions and promoter regions have declared that there are number of single nucleotide polymorphisms in promoter region that may influence plasma glucose concentration, G2h and insulin levels (17-19).

Two of the most frequently used hypoglycemic agents for management of diabetes are Glibenclamide (Glyburide, Micronase, Diabeta, Glynase) (20) from Insulin secretagogues (Sulfonylureas) class and Metformin (Glucophage, Glumetza) (21) from Insulin sensitizers (Biguanide) class.

Sulfonylureas agents close adenosine triphosphate–sensitive potassium (KATP) channels on the pancreatic β cells, consequently depolarize them, and induce insulin release (22).

Metformin is one of the most efficacious oral hypoglycemic agents and is associated with favorable clinical outcomes. Metformin enhances insulin sensitivity and decreased hyperinsulinemia, leading to significant decreases in plasma leptin, cholesterol, triglycerides, and free fatty acid levels (23-25).

Recently, some studies emphasized on the importance of single nucleotide polymorphisms variation in response to antidiabetic drugs. For instance, studies have scrutinized the role of variations in genotypes of some adipokines like adiponectin in response to antidiabetic drugs in type 2 diabetic patients (26, 27).

Regarding reported influence of visfatin gene polymorphism on glucose homeostasis, we aimed to evaluate the effect of SNP (rs2110385) in the promoter region of visfatin gene on insulin resistance, insulin sensitivity and response to oral antidiabetic agents.

Methods

Study population

Subjects were recruited from an outpatient clinic of Dr. Shariati Hospital (an affiliated educational hospital of Tehran University of Medical Sciences, Tehran, Iran) from January to June 2008. The diagnosis of T2DM was based on the World Health Organization criteria (28). Inclusion

criteria were age≥40 years, BMI (Body mass index) ≥25 kg/m2 and at least 2 years of diagnosis of type 2 diabetes and oral treatment with antidiabetic drugs as long as one year. Exclusion criteria were history of type1 diabetes, any chronic disease other than T2DM and its complications and insulin therapy.
Informed written consent was obtained from all subjects before their participation in the study. The study protocol was approved by ethics committee of EMRC (Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences).

Laboratory measurements
The peripheral blood were drawn after 10-12 hour fasting. HbA1C measured using HPLC (High pressure liquid chromatography) exchange Ion method (DS5 England); FPG (fasting plasma glucose) was performed by GOD/PAP method. OGTT (Oral glucose tolerance test) was performed according to the World Health Organization standard protocol (29). Participants were administered a standardized glucose solution of 75 gr glucose in 250 ml of water. Blood samples were taken after 120 min to measure plasma glucose concentrations by utilizing the GOD/PAP and Randox method laboratory kits. Serum visfatin concentration was determined by ELISA method (Human visfatin ELISA kit, AdipoGen Pharmaceuticals, Belmont, Seoul, Korea), minimum detectable concentration was 30 pg/ml, IntraCV was 4.3 % and InterCV was 7.5 %. Serum insulin concentrations were measured by ELISA method (Human insulin ELISA kit, DRG Pharmaceuticals, GmbH, Germany) minimum detectable concentration was 1.76 µlU/ml; Intra CV was 2.19% and InterCV was 4.4%.

HOMA and QUICKI indices calculation
Insulin resistance (IR) was calculated by homeostasis model assessment (HOMA). The HOMA-IR was calculated as following equation: HOMA-IR= (Fasting Plasma Glucose×Fasting Plasma Insulin)/22.5 (30). QUICKI was calculated as equation: ISQUICKI= 1/ [log (fasting insulin) +log (fasting glucose)] (31).

Extraction of genomic DNA
DNA extraction was carried out using FlexiGen Kit (QIAGEN Inc. Valencia, CA) from whole blood according standard protocol. The extracted DNA was stored at 4°C until it used for PCR and RFLP analysis.

Genotyping
Genomic DNA from all subjects was analyzed for the presence of the G or T nucleotide at -4689G/T of the visfatin gene by a designed visfatin genotyping kit.

Statistical analysis
Results are reported as mean ± SD. All of the statistical analyses were performed using the SPSS version 15 software. Student T-test was used to compare quantitative variables. Chi-square was used For comparing of qualitative variables. Also ANOVA was used for comparing the quantitative variable in different genotypes. P−value less than 0.05 was considered as statistically significant.

Results

Ninety four type 2 diabetic patients participated in this study including 20 (21.2%) men and 74 (78.8%) women. Table 1 demonstrates demographic and biochemical characteristics of participated patients.
Fifty patients (53.19%) were classified as poor glycemic control (HbA1C>7%) and others (46.81%) were in good glycemic control status. Genotype frequency of homozygous major allele (GG), heterozygous (GT) and homozygous minor allele (TT) were 31.2%, 50.5% and 18.3%, respectively. Also, 60.63% of patients had G allele and 39.37 % had T allele.
Among patients with G allele, 54.38% were in poor glycemic control and others

were in good glycemic control status. Regarding T allele, 51.35% were in poor glycemic control and others were in good control; nonetheless, there was no significant difference between them.

There was no significant gender difference in poor control group, as included 50% of men and 55.4% of women (P=0.3).

Approximately 62.9% of poor control patients had BMI more than 30 and 49%, had BMI less than 30 (P=0.05). Just about 72.4% of patients with GG genotypes had BMI less than 30 ,but in TT and TG genotypes, BMI values were 52.9% and 55.3%, respectively (P=0.07). Laboratory findings and dosage of antidiabetic medications of patients with different genotypes are shown in Tables 2 and 3.

The required dose of glibenclamide for adjustment of glucose homeostasis was lower in genotype GG compared to others, but there was no difference in required dose of metformin between various genotypes (Table 3).

As shown in figure 1, the number of glibenclamide tablets used by patients with GG genotype was the lowest among different genotypes.

We found no significant difference in FBS, G2h, HbA1C levels, Fasting insulin concentration, and HOMA and QUICKI indices between various genotypes as well as the number of metformin tablets used by patients (Tables 2 and 3).

Table 1. Demographic characteristic of participants in study

variables	Values
Age (years)	55 ±10
Duration of T2DM diagnosis (months)	57±45
BMI(kg/m2)	29.3±3.3
FBS (mg/dl)	159.07±68.29
OGTT(mg/dl)	199.69±83.90
Hb A1C (%)	7.46±1.78
HOMA	5.35±2.82
QUICKI	0.50±0.06
Fasting Insulin (µlU/ml)	14.26±6.82
adiponectin(µg/ml)	8.12±4.62
Visfatin(ng/ml)	14.02±14.69
Mean intake energy (K cal.)	1998.92±1566.86
Dose of metformin (mg/day)	565±680
Dose of glibenclamide (mg/day)	11.2±8.25

Data are means±SD, BMI: Body Mass Index, BMD: Bone Mineral Density, FBS: Fasting Blood Sugar

Table 2 . Characteristics of patient according to genotype

Characteristics	Genotypes *		
	TT	GG	GT
Age (years)	57±8	58±9	53±10
Time duration of T2DM (months)	61±38	52±51	60±45
BMI(kg/m2)	29.77±4.76	28.55±2.91	29.74±3.87
FBS (mg/dl)	162.40±61.32	160.76±81.36	156.80±62.76
2hPP (mg/dl)	185.51±29.46	195.60±96.20	206.46±90.44
Hb A1C (%)	6.90±0.48	7.75±2.11	7.46±1.80
HOMA	4.02±1.46	5.09±2.58	5.98±3.16
QUICKI	0.52±0.05	0.51±0.07	0.49±0.06
Fasting Insulin (µlU/ml)	5.53± 11.41	5.88 ± 13.52	7.50± 15.74
Visfatin(ng/ml)	18.45± 15.85	13.75 ± 14.79	12.88 ±14.13

Data are means±SD, BMI: Body Mass Index, BMD: Bone Mineral Density, FBS: Fasting Blood Sugar, PP: Post Prandial, * differences were not significant (P>0.05)

Table 3. Amount of antidiabetic agents for glucose homeostasis in different genotypes

Amount of antidiabetic agent	Genotypes		
	TT	GG	GT
Mean dose of glibenclamide†	11.07±9.39	9.23 ± 8.20	15.62±9.67
Mean dose of metformin	500 ±392.23	384.61±605.10	637.50 ±911.02
Number of total antidiabetic drugs*†	3.21±2.41	2.61±2.35	4.40±3.30

Data are means±SD, * metformin + glibenclamid, †P-values were significant (<0.05).

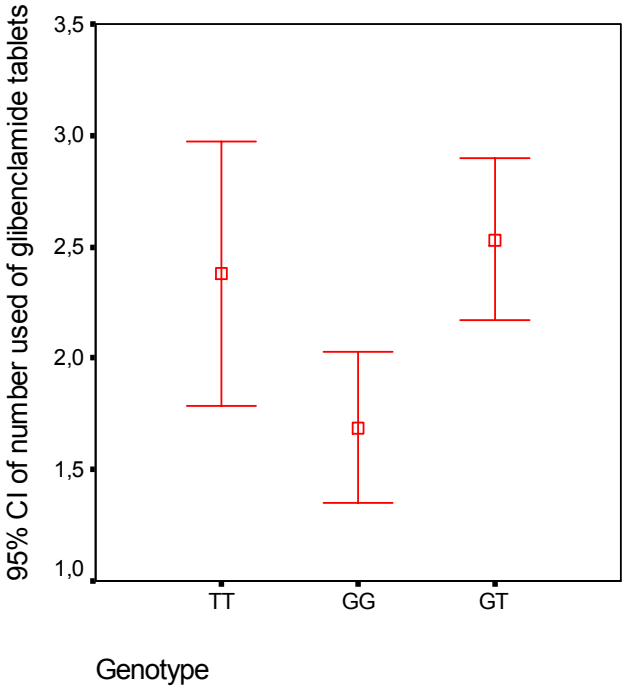

Figure 1. Number of glibenclamide tablets for glucose homeostasis in different genotypes

Discussion

Baileyet al. reported homozygous major allele (GG), heterozygous (GT), homozygous minor allele (TT) frequencies as 33.6%, 50.2% and 16.2%, respectively in the Quebec Family Study that is comparable to our results (32). In favor to our findings, they didn't find any significant difference in fasting glucose levels between three genotypes.

Participants with TT genotype had lower fasting insulin levels and consequently, used more quantities of glibenclamide for controlling blood sugar; conversely, there were no significant differences between markers of blood sugar control (FBS, G2h and HbA1C) in GG genotype; we concluded that the differences in genotypes may play an important role in required amount of medications.

Several studies have investigated the importance of various cytokines and related genotypes including adipocytokines in glucose homeostasis and patients response to antidiabetic drugs.

Zhang et al. (33) showed the effect of single nucleotide polymorphism (SNP) + 45 of the adiponectin (ADIPOQ) gene on the response to therapy with rosiglitazone maleate in patients with type 2 diabetes. They evaluated 103 newly diagnosed drug-free type 2 diabetic patients and treated them with rosiglitazone maleate (4 or 8

mg/d) for 24 weeks and demonstrated a significant difference in the response rate to treatment between genotypes. Their survey showed that genetic variation of adipokines can affect the response to antidiabetic agents.
Kang et al. (34) also examined the effects of rosiglitazone on adiponectin and plasma glucose levels in relation with common adiponectin gene (ACDC) polymorphisms. They showed that SNP45 was associated with reduction in the fasting plasma glucose (FPG) levels and the HbA1c values under rosiglitazone treatment. Regarding SNP276, there was less reduction in the FPG levels for the GG genotype than for the other genotypes.
Recently, Becker et al. (30) revealed the effects of SNP rs2289669 in the SLC47A1 gene on reducing in HbA1C after metformin treatment. Liu et al. (35) investigated the effects of genetic polymorphism of leptin and TNF-alpha on rosiglitazone response in Chinese patients with type 2 diabetes. They assessed 245 patients with T2D and 122 healthy volunteers for leptin G-2548A and TNF-alpha G-308A genotypes by using PCR-RFLP and reported TNF-alpha G-308A polymorphism may be contributed with therapeutic efficacy of rosiglitazone in T2DM patients.
Sun et al. (26) assessed the association of adiponectin allele 45T/G and -11377C/G polymorphisms with type 2 diabetes and rosiglitazone response in Chinese patients and declared lower rosiglitazone effect on FPG, PPG, HOMA-IR in patients with -11377CG+GG heterozygote genotype as compared with -11377CC homozygote genotype. Recent studies (36, 37-39) were demonstrated the association between PPARγ and PPARα and effects of antdiabetic agents as possible mechanism. Regarding the proposed role of PPAR in visfatin pathway (38), we suggest that visfatin may influences insulin secretion and consequently, patient responses to antidiabetic agents.
We observed significant differences between various genotypes and response to glibenclamide in T2DM patients but not for metformin.
For reasons not completely known, the resulting differences in drug effects were much less pronounced. Even so, visfatin genotype-based dose adjustments may reduce the required dose of drug. The magnitude of how doses might be adjusted can be derived from pharmacokinetic studies.
Hence pharmacogenetic variability plays an important role in the pharmacokinetics of oral antidiabetic drugs; however, to date, the impact of this variability on clinical outcomes in patients is not carefully investigated and further prospective studies on the clinical advantages of visfatin genotyping are warranted.

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