VISFATIN G-948T POLYMORPHISM IN EGYPTIAN TYPE2 DIABETES

Hannan A Rehiem [1], Randa F Abd Al Salam[*1], Tarek H Gado [1] and Lila A Rashed[2]

[1]Department of internal medicine[2]; Department of clinical Biochemistry, Faculty of Medicine, Cairo University, Cairo. Egypt.
randa.fayez@hotmail.com*

Abstract: Visfatin is a newly discovered adipokine found in abundance in visceral fat. It lowers plasma glucose in human and mice. In this study, we investigated the role of genetic variant (G – 948 T) of visfatin on serum visfatin and biochemical markers in type 2 diabetic patients. In a case control study 40 diabetic obese type2 compared to 20 healthy controls age and sex matched. Laboratory and anthropometric measurements were included fasting blood glucose, HbA1C, lipid profile, serum visfatin serum insulin, Body Mass Index (BMI), waist hip ratio. Visfatin G – 948Tgene polymorphism was performed using the real-time PCR method. Our results showed significant correlation between visfatin BMI, waist, WH ratio, fasting glucose, fasting insulin, HOMAIR, cholesterol, triglycerides, LDL but negative correlation with HDL in obese diabetics. G-allele had higher BMI, fasting blood glucose, cholesterol, triglyceride, HOMAIR, fasting insulin than T-allele carrier however not statistically significant. In conclusion: Variants of visfatin allele might be responsible for changes in visfatin expression and biochemical markers in unrelated Egyptian type 2 diabetic obese patients, visfatin G-948T polymorphism G allele may account for the development of insulin resistance. [Journal of American Science 2010;6(10):509-514]. (ISSN: 1545-1003).

Key words: Visfatin ; Gene polymorphism ;Diabetes; Obesity

1-Introduction:

The messenger RNA expression of the adipokine visfatin was known as Pre-B-Colony-enhancing Factor 1(PBEF 1) increases during the development of obesity and its plasma level strongly correlates with the amount of visceral fat.[1] Visfatin was reported to imitiate the action of insulin through a distinct binding site on the insulin receptor, resulting in lower blood glucose level however its concentration in plasma is much lower than insulin.Visfatin is higher two folds in type 2 diabetic patients than non diabetic control. Suggesting the role of visfatin in glucose metabolism and in the pathogenesis of type 2 diabetes.[1]

It has been reported that variations of the genes for other adipocytokines, can influence the risk of type 2 diabetes but the relation between, visfatin gene polymorphism and type 2 diabetic remain un known[3]

The relation of visfatin plasma levels, obesity and type 2 diabetes mellitus have been inconsistent. It is possible that genetic variation in PBEF1 contributes to these conflicting results.

The aim of this study is to investigate if single nucleotide polymorphism (G-948) variant allele might be responsible for changes in visfatin expression and biochemical markers in unrelated Egyptian type 2 diabetic obese patients in a case control study.

2-Research Design and Methods:

2.1. Patients:

60 unrelated Egyptians were included in the study with age ranging from 31-72.Forty diabetic patients BMI>30 recruited from outpatient clinic at Kasr El-Aini University Hospital from November 2009 to May 2010.

Twenty healthy volunteers matched for age and sex (control group) with no family history of type 2 diabetes mellitus. All the participants gave their oral consent for approval to participate in the study

Exclusion Criteria:

1. Acute and chronic inflammatory conditions.
2. Sepsis.
3. Renal disease.
4. Liver disease.
5. Congestive heart failure.

All of these groups were excluded from the study due to increase of serum level of visfatin leading to false positive results.

2. 2All patients are subjected to: Complete history taking. Detailed physical examination.,Fundus examination,. Body mass index (BMI) as calculated by dividing the subject's weight by the square height (BMI = weight in kilograms / height in meters^2).

Waist Hip Ratio (WHR): Waist and hip circumferences were measured to nearest 0.1 cm, waist measured at narrowest point between lowest

ribs; hip measured at upper most lateral border of right iliac crest

Laboratory investigation done after an overnight fast Fasting blood glucose, Serum creatinine, blood urea nitrogen, total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein. Homeostasis Model Assessment of insulin resistance (HOMAIR) test was calculated from fasting insulin and fasting glucose by the following equation:

HOMAIR = fasting insulin (uU/ml) x Fasting glucose (mmol/liter)/ 22.5

Visfatin serum level.

Samples were drawn after overnight fast,fasting plasma glucose,total cholesterol,triglyceride and HDL were measured by enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics GmbH (Hamburg, Germany).

LDL calculated by Friendwald's formula.The serum basal insulin level was determined by the coated tube method (DPC,Los Angeles,CA,USA)).

For Visfatin and insulin measurements samples were kept at -80c.

Plasma visfatin levels was determined by ELISA method(Human visfatin ELISA kit,phoenix pharmaceuticals,Belmont,CA, USA).

The serum basal insulin level was determined by the coated tube method (DPC, Los Angeles, CA, USA)).

2.3Genotyping:

The TaqMan allelic discrimination assay (Custom TaqMan SNP Genotyping Assay; Applied Biosystems). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7500 (95 C for 10 min and then 95 C for 15 sec and 62 C for 1 min for 38 cycles), and fluorescence was detected on an ABI PRISM 7700 sequence detector (Applied Biosystems). all genotypes matched initial designated molecular study of single nucleotide polymorphisms (SNP) in the visfatin gene (948 G>T) for genotyping ,genomic DNA was extracted and then SNP genotyping was performed using the rapid and reliable allele-specific real-time PCR method .The single nucleotide variations were discriminated by the 3end of the allele-specific primer.Blood was collected under sterile a septic technique and placed in a sterile EDTA tube .DNA was extracted from whole blood using DNA extraction kit (BioRad,Italy) The average DNA concentration was determined by absorbance at 260 nm using perkin elmer spectrophotometer and The integrity of the DNA was checked electrophoresis on 0.8 % agarose gel with an ethidium bromide. the affected exon considered was amplified using genotypes .

2.4. Statistical Methods

Data was coded and entered using the statistical package SPSS version 15. Data was summarized using mean and standard deviation for quantitative variables and number and percent for qualitative variables. Comparison between groups was done using chi-square tests for qualitative variables while analysis of variants with post HOC tests. Independent sample T test were used for normally distributed quantitative variables. While non parametrical Kruskal- Wallis test and Mann – Whitney test were used for quantitative variables not normally distributed correlations were done to test for linear relations between quantitative variables, p-values less than 0.05 were considered as statistically significant. The frequencies of alleles or genotypes were compared by x^2 analysis,differences of continous variables among genotypes were evaluated by one way ANOVA.

3. Results

The Characteristics of the study group and the control are given in Table (1).

Age – sex distribution were similar among the two groups.

Table (1) Patients Characteristics in our Study.

	Diabetic Obese	***Control***	**P.**
Sex (male/female)	40 (5/32)	20 (8/12)	0.022
Age (y)	54.7	43.7	*<0.001
BMI (Kg/m^2)	31.7	23.4	*<0.001
Waist (cm)	93.2	74.8	*<0.001
WH Ratio	0.9092	0.872	*<0.001
Systolic B.P	136.1	120.25	*<0.001
Diastolic B.P	83.25	76.5	*<0.001
Fasting glucose (mmol/L)	7.8	3.9	*<0.001
Fasting insulin (uU/ml)	20.8	11.0	*<0.001
HOMAIR	7.4	1.9	*<0.001
Cholesterol(mg/dl)	326.3	194.3	*<0.001
Triglyceride	96.9	73.9	*<0.001
LDL (mg/dL)	83.9	62.5	*<0.001
HDL (mg/dL)	38.4	49.9	*<0.001
Visfatin(ng/ml)	13.1	9.4	*<0.001

Data are median.

* P-value <0.05 is significant.

HOMAIR indicates homeostasis model assessment index of insulin

We analyzed PBEF1, single nucleotide polymorphism G – 948T in cases and control. There were no significant differences between cases and controls concerning allele frequencies (P=0.173) neither with genotype distribution (P=0.352) table (2).

Table (2) Comparison of genotype and allele distribution of G-948T for diabetic subjects and control.

	Allele Frequency		*P-value*	*Genotype distribution*			P
	G	T		T/T	T/G	G/G	
T_2DM (n=40) **Table (4).**	37	43	0.173	16	11	13	0.352
Control (n=20)	13	27		10	7	3	

G-allele had higher BMI, fasting blood glucose, cholesterol, triglyceride, HOMAIR, fasting insulin than T-allele carrier however not statistically significant, shown in table (3)

Table 3) Genotypes of G-948T gene polymorphism and clinical data of diabetic patients.

	T/T	T/G	G/G	T/T (VS) GG (P-value)	T/T (VS) TG (P-valueee)
BMI (kg/m^2)	30.962	31.1	33.3	0.987	1
Fasting glucose (mmol/L)	7.0	7.3	9.0	0.013	1.0
Fasting insulin (uU/ml)	19.0	20.2	23.6	0.169	1
Total cholesterol (mg/dL)	319.5	321.5	338.9	0.893	1
Triglyceride (mg/dL)	94.5	93.9	102.5	0.471	1
HDL (mg/dL)	39	39	37.3	1	1
HOMAIR	5.9	12.65	9.6	0.012	1
Visfatin(ng/ml)	13.03	12.65	13.59	0.885	1

We next analyzed the correlation between plasma levels of visfatin anthropometric and laboratory parameters in diabetics. There was a significant Correlation between visfatin BMI, waist, WH ratio, fasting glucose, fasting insulin, HOMAIR, cholesterol triglycerides LDL Negative correlation with HDL, shown in table (4)

Table (4) Correlation between visfatin and anthropometric parameters in diabetics

	visfatin Level
Age	r = -0.58 p = 0.723
Duration of diabetes	r = -0.123 p = 0.451
BMI	r = 0.555 * p <0.01
Waist	r = 0.49 *p = 0.001
WH ratio	r = 0.108 p = 0.505
Systolic B.P	r = 0.223 p= 0.167
Diastolic B.P	r = 0.128 p = 0.432
Fasting glucose(mmol/L)	r = 0.331 p = 0.037
Fasting insulin(uU/ml)	r = 0.542 *p<0.01
HOMA IR	r = 0.515 *p <0.001
Cholesterol(mg/dl)	r = 0.534 *P <0.01
Triglyceride(mg/dl)	r = 0.62 *p<0.02
LDL(mg/dl)	r = 0.432 *p = 0.005
HDL(mg/dl)	r = -0.514 *p = 0.001

*p value significant if<0.05

4. Discussion

Obesity , abdominal obesity in particular is strongly associated with dyslipdeamia and increased risk for type 2 diabetes and metabolic syndrome [1,2] Visfatin is a visceral adipokine playing a role in diabetes and metabolic syndrome , with potentially important effects on glucose metabolism and atherosclerosis[16]. visfatin is a 473-amino acid protein with a molecular mass of 52 kd mainly expressed in liver,skeletal muscle adipose tissue and bone marrow. It has been linked to several inflammatory conditions, beta cell function, and cardiovascular disease. The growing number of publications on the subject will give a better insight about the complex intercorrelation between adiposity and glucose metabolism[4,8,9]

Visfatin have insulin sensitizing properties through a distinct binding site on the insulin receptor resulting in lowering blood glucose level [17]

It was shown to stimulate glucose uptake into adipocytes and muscle cells and suppress glucose release from hepatocytes in vitro.Moreover, Fukuhara showed that visfatin induced phosphorylation of signal transduction proteins downstream of insulin receptor[1].Although Berndt, could not show significant differences in visfatin m RNA expression ,BMI and percent body fat .The findings of Fukuhara and Berndt suggest a potential relationship between visfatin and glucose homeostasis or adipocyte proliferation and indicate the possibility that abnormalities of the visfatin gene could contribute to the genetic predisposition for diabetes and could play a role in the association between obesity and type 2 D.M[1,17] . However its concentration in plasma is much lower than insulin[1].

The variation of adipocytokines gene mainly visfatin can influence the risk of type 2 diabetes but

the relation between visfatin gene polymorphism and type 2 diabetes remain inconsistent [4,13,17,18,19]

The physiological mechanism by which visfatin 948G→T polymorphism affect changes in glucose metabolism ,body fat content ,and visceral/subcutaneous fat ratio of mRNA expression is unclear, however increased amount proinflammatory cytokines such as TNFα or IL-6 may explain increased level of visfatin in visceral fat[5]

To clarify the relation between visfatin 948-T and metabolic syndrome we studied the role of visfatin gene G-948-T polymorphism in the predisposition to metabolic syndrome. Our results showed that G-allele carrier had higher BMI, WHR,fasting blood glucose , insulin , lipid profile , HOMAIR and Visfatin level than T-allele carrier however not statistically significant.

Various studies showing results similar to our study[6,10,11,12,14,15]

Variants in the promoter region of PBEF1 have earlier been found to correlate with fasting insulin, fasting glucose. (rs 9770242, G-948T and rs 1319501) indicating a possible association with insulin resistance.[6,10,11,12]

Several reports have shown a positive association between visfatin and HDL cholesterol in various populations. These findings are likely a reflection of differences in insulin sensitivity between the subjects resulting in typical dyslipdemia, insulin resistance and metabolic syndrome. Indicating a possible association between visfatin and insulin resistance[3,5,6,7,19] .Our study showed a negative correlation between visfatin and HDL(r=-0.541,p=0.001)

Bottcher et al, investigated the role of visfatin gene in pathophysiology of obesity in type 2 diabetes in a case control study (503 diabetec subjects and 476 healthy controls)seven single nucleotide polymorphisms were identified three SNPS (rs 9770242, - 948G→T, rs 473030153) .That were representatives for their linkage disequilibrium groups genotyped in Caucasians from Germany with a wide range of body fat distribution and insulin sensitivity. Visfatin mRNA expression was positively associated with all three genetic polymorphisms ($P < 0.05$) More over the 948 G→T variant was associated with increased 2h – plasma glucose and fasting insulin concentrations($P < 0.05$) in non diabetic subjects concluding that genetic variation in the visfatin gene may have a minor role in the development of obesity and type 2 D M[13]

However Bottcher result were contrary to our findings as we found positive correlation between visfatin and fasting glucose,fasting insulin ($p=0.037$, $p<0.01$ respectively)

In study done by Johnason et al, on obese Scandinavian subjects (n = 235) in a case control study. In this study, they investigated if 2 single nucleotide polymorphisms, ts 4730153 and G – 948T were associated with obesity and whether they influence the (m-RNA) levels of visfatin in visceral or subcutaneous adipose tissue (VAT ad SAT). They found that obese carriers of visfatin G-948T G variant allele had significantly higher level of HDL cholesterol (GG 1.1 (0.97 – 1.3) mmo/L GT + 77, 1.3 (1-1.5) mmo//L p=0.02 However neither rs 4730/153 nor G – 948T had any major impact on any of the obesity related , no difference in mRNA expression between phenotypes in visceral and subcuteanous adipose tissue .However a significant correlation was observed between body Mass index and PBEF1 M-RNA expression in SAT ($r = 0.37$, $P = 0.03$) but not in VAT ($r = 0.26$, $P = 0.12$).In Conclusion, PBEF1 G-948T is associated with increased HDL cholesterol, but genetic variation in visfatin does not seem to have a major impact on the development of obesity or on the expression of the gene[14]

Our results were similar in a way to Johnason et al, G-allele had higher BMI, fasting blood glucose, cholesterol, triglyceride, HOMAIR, fasting insulin than

T-allele carrier however not statistically significant

Jian et al, determined association between the visfatin gene, type 2 diabetes and other metabolic and anthropometric parameters, they found that visfatin gene was associated with increased glucose and lipid profile. They explained their finding by the location of The visfatin gene at 7q22.2 which has been reported as a linkage region with metabolic syndrome, BMI HDL and triglycerides[15].

The relation of Visfatin genotyping with diabetes and metabolic syndrome in different populations was published in different studies as that of Tokunaga et al,Mirzaei et al.

Tokunaga et al, investigated the role of genetic variation in the visfatin gene in the pathophysiology of type 2 diabetes in Japanese subjects. The 11 exons, and the promoter region of the visfatin gene were screened for single nucleotide polymorphisms (SNPs) by PCR-direct sequencing. Tokunaga et al found SNPs in the promoter region (SNP - 1535T>C), exon 2 (SNP + 131C>G, Thr44Arg), and exon 7 (SNP + 903G>A). The allele and genotype frequencies of these SNPs showed no significant differences between (448) diabetic and (333) control subjects. However, the -1535T/T genotype was associated with lower serum triglyceride levels (T/T vs. T/C + C/C ($p = 0.015$) and T/T vs. C/C ($p = 0.043$)) and higher HDL-cholesterol levels (T/T vs. C/C, $p = 0.0496$) in the nondiabetic

subjects. Reporter gene assay of 3T3-L1 adipocytes revealed that the promoter activity of -1535T and -1535C were similar, suggesting that the observed association may reflect linkage disequilibrium between -1535T>C and causative variations of the visfatin gene.[6]

Mirzaei et al,investigated the role of -4689G/T promoter variant of the visfatin gene on serum visfatin concentration and biochemical markers in type 2 diabetic(93)diabetic Iranian patients (FBG,OGTT,HbA1C,lipid profile ,fasting serum visfatin,serum insulin,weigh,BMI,WHR) Genotyping for visfatin gene was performed by using the PCR-RFLP method

Results :significant difference in levels of lipid profile , fasting insulin,among various types of visfatin genotypes (TT,GG,GT) A significant correlation between circulating levels of visfatin and weight,BMI,hs-CRP and fasting insulin[20]

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5. Conclusion:

Our results showed significant correlation between visfatin, BMI, waist, WH ratio, fasting glucose, fasting insulin, HOMAIR, cholesterol, triglycerides, LDL but negative correlation with HDL in obese diabetics.

G-allele had higher BMI,fasting blood glucose, cholesterol, Triglyceride, HOMAIR, fasting insulin than

T-allele carrier however not statistically significant.

In conclusion. visfatin G-948T polymorphisim may account for the development of insulin resistance .

6. Limitation:

We didn't study other SNPS (Single Nucleotide Polymorphisms) reported in Caucasian subjects including SNPS-1458T>C, SNP – 1001 G>T, SNP – 520G>A, SNP- 422G>A ,SNP – 422G>T, SNP – 194C>T, SNP 129C>T which might give a better linkage of visfatin gene polymorphism to diabetes and metabolic syndrome. The limited sample size in the present study means that longitudinal studies with more patients will be needed.

Correspondence authors:
Randa F Abd EL Salam
Department of Internal Medicine, Faculty of Medicine,Cairo university
Telephone: 002-02-27634980
Cellular phone: 002-0101407278
E-Mail:randa.fayez@hotmail.com

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