Genetic Variations in Visfatin and Resistin Genes in Egyptian Type 2 Diabetic Patients With and Without Complications

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Abstract

Diabetes mellitus is one of the main threats to human health in the twenty-first century. Visfatin and resistin are adipokines which have been implicated in the pathogenesis of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) complications. Several genetic studies have shown inconsistent results regarding association of visfatin and resistin gene polymorphisms with T2DM and CVD complications.

Subjects and methods: Here we investigate whether visfatin -948G/T and resistin -420C/G polymorphisms are associated with T2DM, its CVD complications and serum adipokine levels in 90 Egyptian diabetic patients (44 without CVD and 46 with CVD) along with 60 healthy control subjects. Polymorphisms of visfatin -948G/T and resistin-420C/G genes were detected by Real time-PCR and PCR-RFLP, respectively. Serum visfatin and resistin were measured by ELISA. Results: Higher frequencies of visfatin -948G/T and resistin -420C/G were observed among T2DM patients compared to controls. Furthermore, the frequencies of these genotypes were significantly higher in diabetic patients with CVD than in those without CVD. Both -948G/G and -420G/G genotypes and G alleles were significantly associated with T2DM and CVD in Egyptian diabetic patients. Moreover, serum visfatin and resistin levels were markedly elevated in T2DM patients with the highest values observed in G/G genotypes among diabetic patients with CVD. In addition, positive correlations were observed between plasma adipokine levels and CVD risk factors. In conclusion, our data suggest that genetic variations in visfatin -948G/T and resistin -420C/G may contribute to the disposition for T2DM and its CVD complications in Egyptian patients.

Keywords: T2DM, CVD, Visfatin -948G/T, Resistin -420C/G, Polymorphisms.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that causes significant morbidity and mortality. It affects about 258 million adults worldwide and is expected to increase to 439 million adults by 2030, indicating a growing burden of diabetes, particularly in developing countries [1]. Insulin resistance is a major contributor to the pathogenesis of T2DM and plays a crucial role in the development of its associated complications [2, 3]. It is now well recognized that adipose tissue is an active endocrine organ that is known to express and secrete a variety of adipokines [4]. Overwhelming evidence suggests that the release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic sub-inflammatory state that may represent the link between insulin resistance, T2DM and cardiovascular disease (CVD) [5-7]. CVD including atherosclerosis, vascular disease and coronary heart diseases are considered the main cause of death in developed countries, are mainly. Risk factors for the development of atherosclerosis include type 2 diabetes mellitus (T2DM), hypertension, hyperlipidemia and smoking [8]
and diabetic patients with CVD have been shown to have higher mortality and morbidity compared to non-diabetic subjects [9].

Visfatin a novel adipokine isolated by Fukuhara et al [10] is predominantly secreted from the visceral fat of humans and mice and was found to be identical to the previously identified growth factor, pre-B cell colony-enhancing factor (PBEF). It also functions as a nicotinamide phosphoribosyl transferase (Nampt), the rate-limiting enzyme in the salvage pathway of nicotinamide adenine dinucleotide biosynthesis. Moreover, it is involved in the modulation of inflammation and innate immunity [11]. Visfatin binds and activates the insulin receptor, although at a site distinct from insulin, it exerts several insulin-mimetic actions in various cell lines and lowered plasma glucose levels in mice [10, 12]. However, human studies have shown inconsistent results regarding associations between visfatin and insulin resistance, diabetes and its complications [13, 14]. It is possible that genetic variation in visfatin contributes to these conflicting results.

The visfatin gene is located on chromosome 7q22.2, which is composed of 11 exons and 10 introns, spanning 34.7 kb of genomic DNA [15] and considered as a candidate gene for human T2DM [16]. Several single nucleotide polymorphisms (SNPs) in the visfatin gene were reported to be associated with insulin resistant [16, 17], low-grade inflammation, high risk of T2DM [18-20] and CVD [21-26]. But the relation between visfatin gene polymorphism and T2DM remain inconsistent [14, 24, 27]. One of the most frequently studied SNP is -948G/T (rs978040) which also known as -948C/A [18, 20] which is in perfect or moderate linkage disequilibrium (LD) with The function of visfatin related to glucose homeostasis and insulin resistance in type 2 diabetic patients is not consistent published on the relation of visfatin -948G/T SNP and T2DM with the risk of CVD. We investigate the potential relation of visfatin -948G/T SNP and T2DM with the risk of CVD in an Egyptian population.

Resistin (or resistance to insulin) is another adipocyte-derived peptide, belongs to a family of cysteine-rich secretory proteins. In human resistin is primarily expressed in monocytes or macrophages. Thus, adipocytes contribute to only a small fraction of resistin production [28]. The function of resistin related to glucose homeostasis and insulin resistance in type 2 diabetic patients is not consistent [29, 30]. However, some studies have reported increased resistin expression levels in T2DM and insulin resistance [31-34]. Meanwhile, it is considered to be involved in the pathogenesis of atherosclerosis, acute coronary syndrome [35] and CVD [33, 36-40]. In addition to, resistin has been found to have possible roles in the development of endothelial dysfunction, thrombosis, angiogenesis, inflammation and smooth muscle cell dysfunction [37]. Therefore, the study on genetic variation of RETN (the gene coding resistin production in human), has become the target of several investigations about identification of involved mechanisms in pathogenesis of T2DM and CVD.
Resistin gene is located on chromosome 19p13.3 [41], and up to 70% of the variation in serum resistin levels can be explained by genetic factors [42]. There are several single nucleotide polymorphisms in the resistin gene have been described. One of the most frequently studied polymorphisms is -420C/G. The SNP on resistin gene promoter -420C/G (rs1862513), as one of the most commonly studied polymorphisms, was reported to be associated with serum resistin level [28, 32, 43], insulin resistance, T2DM [28, 32, 43-46] and CVD [32, 43, 45, 47-49]. However, not all reports have consistently produced these findings [50-53]. Therefore, we aimed to investigate the possible associations of resistin -420 and gene polymorphisms with T2DM, and to detect whether these polymorphisms are associated with CVD in T2DM. Also, clarifying the impact of these polymorphisms on clinical, biochemical parameters and serum resistin level were of particular interest in our study.

2. Methods

2.1. Subjects

A total of 120 participants (44 male and 76 female) were enrolled in the study: 90 patients with T2DM and 30 age and sex matched non-diabetic healthy control subjects. All participants gave their informed consent and the study was conducted in accordance with the approval of Ethics Committee of Faculty of Pharmacy, Cairo University, Egypt. Diabetic patients were recruited from admitted patients to internal medicine department of Kasr EL-Eini Hospital, Cairo University. They were classified into group I included 44 T2DM patients without cardiovascular disease (CVD) and group II included 46 T2DM patients with CVD. The definition of CVD was based on the histories of physician-diagnosed ischemic heart diseases (e.g. myocardial infarction, angina or any other clinically evident vascular disease). Non-diabetic controls had fasting blood glucose levels of less than 100 mg/dl, were not suffering any health problems and had a negative history for T2DM and CVD. Both the non-diabetic control group and the non-obese diabetic group were selected to have matching BMI. The characteristics and biochemical data of patients and healthy controls are summarized in Table 1.

2.2. Blood sampling and laboratory assays

About 10 ml of fasting venous blood samples were obtained from all participants. Aliquots of blood were collected on EDTA for estimation of plasma glucose, glycosylated hemoglobin (HbA1c) levels and extraction of DNA. The other portions of blood samples were collected in serum separation tubes for determination of insulin, total cholesterol (TC), LDL cholesterol (LDL), HDL-cholesterol (HDL), triglycerides (TAG), urea and creatinine using standard laboratory methods. Serum visfatin and resistin levels were estimated using enzyme-linked immunoassay kits (Uscn Life Science Inc. USA and WUHAN) and (BioVendor, Laboratorni medicina, a.s European Union) respectively. The homeostasis model
assessment of insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose levels as described by Matthews et al [54].

2.3. Genotyping of polymorphisms

Genomic DNA was extracted from whole blood using the QIAamp® DNA minikit (Qiagene, USA) according to the manufacturer’s instructions.

2.3.1. Genotyping of SNP in visfatin -948G/T

Genotyping of −948G/T polymorphisms in genomic DNA was performed using the TaqMan allelic discrimination assay (Custom TaqMan SNP Genotyping Assay; Applied Biosystems). The primers and reporters sequences were:

forward primer: 5’-GCCCGTTGCTTTTCCTT-3’
reverse primer: 5’-GGTGGAATTCAGTCCTCACAGATAA-3’

VIC reporter: CCTAATTGAACCGAGTATT
FAM reporter, CCTAATTGAACAGAGTATT

The reaction conditions used were: 50 ng of genomic DNA in a total volume of 12.5 µl of reaction mixture containing 0.625 µL of 20X diluted SNP mix, 6.25 µL of 2X TaqMan Universal PCR Master Mix (Perkin-Elmer, Applied Biosystems Division) and nuclease free water. The PCR cycling protocol consisted of 2 min at 50°C; 10 min at 95°C (for Ampli Taq Gold DNA polymerase activation); and 50 cycles of 92°C for 15 s (for melting/denaturation) and 60°C for 1 min (for annealing and extending). Fluorescence (via allele specific VIC and FAM dyes) was measured and genotypes were determined using the Applied Biosystems 7300 System Sequence Detection Software.

2.3.2. Genotyping of SNP in resistin -420C/G

Genotyping of -420C/G polymorphisms in genomic DNA was performed using the PCR and restriction fragment length polymorphism (RFLP). The primers sequences were: forward 5’-TGT CAT TCT CAC CCA GAG ACA-3’, reverse 5’-TGG GCT CAG CTA ACC AAA TC-3’. The reaction conditions used were:

30 ng of genomic DNA in a total volume of 50 µl of reaction mixture containing 100 pmol of each primer, 2 mmol dNTPs, 5µl of 10X reaction buffer with MgCl₂ (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 5 µl 10x Taq polymerase buffer (Promega, Madison, WI, USA) and 2 units Taq DNA polymerase (Promega, Madison, WI, USA). The cycling condition were as follows: first, denaturation at 95°C for 3 min followed by 35 cycle of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. After amplification, a 3µL aliquot of PCR products was digested with 5 U Bbs I (GAAGAC) restriction endonuclease (New England BioLabs, Waltham, MA, USA) in 1.5 µL 10X NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCL, 10 mM MgCl₂ and 1 mM dithiothreitol) and 9.5 µL dH₂O at 37°C for 12 h. The 534 bp PCR products were cleaved into two fragments of 327 and 207 bp in the presence of the C homozygous and three fragments of 543, 327 and 207 bp for heterozygote (CG),
while the G homozygous remained uncleaved showing the 543 bp PCR products. The digested PCR fragments were subjected to agarose gel electrophoresis (2%) and visualized by ethidium bromide staining and UV transillumination (Fig. 1).

2.4 Statistical methods:

Results are expressed as mean ± SD. Kolmogorov-Smirnov test was done to evaluate the distribution of variables. Repeated measured ANOVA (analysis of variance) was used for analysis of more than two quantitative data followed by Tukey for detection of significance and t-test was used for analysis of two quantitative data. Any skewed data were further analyzed by Kruskal-Wallis and Mann-Whitney U test. To compare categorical data, a chi squared (χ²) test was performed. Hardy–Weinberg equilibrium was assessed for both subjects and controls by a χ² goodness-of-fit test. Odds ratios (ORs) of T2DM and CVD complications associated with each genotype with 95% confidence of intervals were calculated by logistic regression analysis assuming dominant mode of inheritance. Logistic regression was also performed to find out the risk factor of GG genotype of visfatin -948G/T and resistin -420C/G in T2DM with CVD compared with T2DM without CVD in relation to clinical and laboratory data. The probability values presented are based on two-sided tests. P-value less than 0.05 were considered statistically significant. All statistical calculations were performed using the computer pro-gram SPSS (Statistical Package for the Social Science; SPSS, Chicago, IL, USA) version 20 for Microsoft Windows. Graph pad prism program version 6.0 was used for drawing figures.

3. Results

3.1 Clinical and biochemical characteristics of the study subjects:

As shown in Table1, there was no significant difference in the age, BMI or sex distribution between the different study groups. Type 2 diabetic patients (T2DM) showed significantly higher levels of FBG, HbA1c, Insulin and HOMA-IR compared with healthy individuals. No significant differences were found in these parameters between T2DM without CVD and T2DM with CVD groups. Among the lipid profile, the levels of TAG, TC, LDL-C and VLDL-C were significantly higher, whereas levels of HDL-C were lower in T2DM patients with CVD than those without CVD. Regarding visfatin and resistin levels, significant differences were observed between T2DM patients and controls. Furthermore, T2DM patients with CVD had significantly elevated levels of visfatin and resistin compared to patients with T2DM without CVD.

3.2 Genotypes and alleles distribution for SNP -948G/T and -420C/G:
The observed genotype distributions of visfatin -948G/T and resistin -420C/G agreed with those expected by Hardy-Weinberg equilibrium in all studied groups (Table 2).

Results presented in Table 3 indicated that the frequencies of visfatin -948GG genotype and G allele were significantly higher in T2DM patients without CVD than controls (31.82% versus 13.33%) and (51.14% versus 30%) respectively. Furthermore, a higher visfatin -948 GG genotype frequency was observed in T2DM with CVD group (50%) than in T2DM without CVD group (31.82%). Additionally, the frequency of G allele of visfatin -948G/T was statistically elevated (68.48% versus 51.14%).

Additionally, Table 3 revealed significantly higher frequencies of resistin -420GG genotype and G allele in T2DM patients without CVD compared to controls (27.27% versus 10%) and (47.73% versus 26.67%) respectively. Moreover, in T2DM with CVD group resistin -420GG genotype frequency was significantly higher compared to T2DM without CVD group (47.83% versus 27.27%) and the frequency of G allele of resistin -420C/G was statistically higher (66.30% versus 47.73%).

3.3 Relation between visfatin, resistin polymorphisms and Type 2 diabetes mellitus:

As indicated in Table 4 the odds ratio of visfatin GG genotype and G allele carrier in T2DM without CVD group were (OR=4.31, CI=1.14-16.3, P=0.032) and (OR=2.44, CI=1.22-4.88, P=0.012) respectively. Likewise, carriers of GG genotype and G allele of resistin were significantly more likely to develop T2DM (OR=4.86, CI=1.14-20.70, P=0.033) and (OR=2.4, CI=1.18-4.88, P=0.016) respectively.

3.4 Relation between visfatin, resistin polymorphisms and CVD in type 2 diabetic patients:

Analysis of the association of SNP of visfatin and resistin with CVD in T2DM patients (Table 4) showed that GG genotype and G allele of visfatin were significantly associated with CVD in T2DM group compared to T2DM without CVD group (OR=3.56, CI=1.10-11.51, P=0.034) and (OR=2.17, CI=1.88-3.97, P=0.012) respectively. Moreover, the GG genotype and G allele of resistin -420C/G were observed to be associated with CVD in T2DM patients compared to T2DM patients without CVD (OR=3.67, CI=1.16-11.56, P=0.027) and (OR=2.15, 1.17-3.98, P=0.014) respectively.

3.5 Relation between SNP -948G/T and serum visfatin levels:

The G/G genotype exhibited significantly higher serum visfatin levels in different study groups (Fig.2). In T2DM patients with CVD, GG genotype carriers showed the highest serum visfatin values (47.46 ± 4.23) followed by GT and TT (36.70 ± 3.40 and 28.66 ± 1.55 respectively). Likewise, GG genotype carriers of T2DM patients without CVD showed more elevated serum visfatin values (35.50 ± 6.96) than GT and TT (29.30 ± 1.96 and 24.39 ± 3.62 respectively). Similarly, controls with GG genotype showed
increased levels of serum visfatin $(21.75 \pm 0.99)$ compared with GT and TT $(17.91 \pm 1.09$ and $13.81 \pm 3.10$ respectively).

3.6 Relation between SNP -420C/G and serum resistin levels:

As demonstrated in Fig. 3, the serum resistin levels were significantly high according to the presence of the G allele in different study groups. The highest serum resistin values $(26.68 \pm 5.55)$ were observed in GG genotype carriers among T2DM patients with CVD. While the CG and CC genotypes showed lower values $(21.04 \pm 1.55$ and $17.86 \pm 0.90$ respectively). Likewise, in T2DM without CVD group, GG genotype carriers exhibited more elevated serum resistin values $(21.08 \pm 2.72)$ than CG and CC $(16.88 \pm 1.56$ and $13.14 \pm 1.04$ respectively). Meanwhile, controls with GG genotype showed increased levels of serum resistin $(10.23 \pm 0.25)$ followed by CG and CC $(8.50 \pm 1.08$ and $6.97 \pm 0.94$ respectively).

3.7 Logistic regression analysis:

A logistic regression analysis was performed to test the association of both visfatin -948G/G and resistin -420G/G with different clinical and biochemical characteristics of the study participants in T2DM with CVD group in comparison to T2DM without CVD as presented in table (5 and 6).

Our results revealed that, the GG genotype of both visfatin -948G/G and resistin -420G/G in T2DM patients with CVD were associated with triacylglycerol, total cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol. They were also associated with the first CVD risk predictor (TC/HDL-C) and the second CVD risk predictor (LDL-C/HDL-C). Moreover, both genotypes seems to be negatively associated with low levels of high density lipoprotein cholesterol, however, their odds ratio were insignificant.

4. Discussion

During the past few years, much attention has been focused on the potential role of adipose tissue in the development of vascular complications of diabetes. This study was designed to explore the association of visfatin -948G/T and resistin -420C/G gene polymorphisms in T2DM patients with and without CVD complications. Considering the distinct ethnic feature of the Egyptian population, the high prevalence of T2DM in them and according to our knowledge, the interrelation between visfatin -948G/T and resistin -420C/G gene polymorphisms in Egyptian T2DM patients with CVD has not been elucidated before our current work. Several association studies revealed the contribution of visfatin -948G/T and resistin -420C/G gene variants to the pathogenesis of T2DM and CVD; however, they were inconsistent.

Visfatin has emerged in in last few years as a novel adipokine playing a role in different fields, including NAD biology, metabolism, and inflammation [23]. In the present investigation, we confirmed
that serum visfatin level increased in T2DM compared with control group and this may be due to impaired visfatin signaling in target tissues that means visfatin resistance resulting in eventual hypervisfatinemia like insulin resistance that results eventually in hyperinsulinemia to overcome this resistance [55]. Also, visfatin has been suggested to act as: an enzyme in the catalysis of the rate-limiting step in the production of NAD$^+$ from nicotinamide, a novel insulin mimetic fat secreted factor and a regulatory factor in pro-inflammatory processes, these elevated levels could attributed to the chronic low grade inflammation present in T2DM [23, 55]. Thus, visfatin may be a compensatory mechanism or a part of the pathophysiology of T2DM [55].

The variation of adipocytokines gene mainly visfatin can influence the risk of T2DM but the relation between visfatin gene polymorphism and T2DM is still a matter of debate [16, 17]. Moreover, we found that the frequencies of visfatin -948GG genotype and G allele were significantly increased in T2DM compared to control group, the odds ratio (OR=4.31, P=0.032, OR=2.44, P=0.012 respectively) suggested an association between the presence of the polymorphism and the prevalence of disease. This agreed with McKenzie [18] and Zhang et al [20] who reported an association of visfatin -948GG genotype with T2DM and higher GG genotype and G allele frequencies in American subjects. However, Rehiem et al [19] showed that visfatin -948G/T polymorphisms may account for the development of insulin resistance in Egyptian population, considering higher frequencies of TT genotype and T allele but there were no significant differences between cases and controls concerning allele frequencies neither genotype distribution.

Surprisingly, Bialy et al [17], Botcher et al [16] and Korner et al [24] demonstrated that GG genotype and G allele frequencies were significantly higher in French-Canadian and German populations respectively, but the same authors did not found an association with T2DM at visfatin -948G/G genotype. Interestingly, Bialy et al demonstrated that, the -948G/T variant was found to be associated with fasting plasma insulin levels, these may indicate that visfatin is involved in other aspects of the dyslipidemia associated with insulin resistance [17]. In addition, Botcher et al reported that genetic variation in the visfatin does not have a major impact on diabetes development and was associated with increased 2h plasma glucose and fasting insulin concentrations concluding that genetic variation in the visfatin gene may have a minor role in the development of and T2DM [16]. In contrast, Paschou et al [27] found no significant differences in allele and genotype frequencies between T2DM and healthy controls and no association with T2DM at visfatin -948G/T genotype in Greek population. Meta-analysis obtained by Saddi-Rosa et al [14] revealed that the relation between visfatin gene polymorphism and T2DM remain inconsistent.

In the present study, we have also found a significant increase in serum visfatin among T2DM with CVD compared to T2DM without CVD. Our results corroborate the finding of Saddi-Rosa et al [26] who confirmed the association of high plasma level of visfatin in CAD in T2DM of the Brazilian cohort without clear explanation of these results. Moreover, Uslu et al [33] who suggest that increased visfatin levels in T2DM may be novel biochemical risk factors for CVD complications among Turkish population. Filippatos
et al [23] examined the association of visfatin with atherosclerosis-related metabolic variables which result in increased CVD risk in England population. In addition, meta-analysis revealed by Chang et al [21] who suggested that the use of visfatin may predict diabetes status, insulin resistance, metabolic syndrome and cardiovascular disease. Romacho et al [56] who showed that visfatin has been proposed as a marker of endothelial dysfunction, an initial and crucial step in progression of the atherosclerotic process. So, supporting a role of visfatin as a potential biomarker of CVD complications associated to metabolic disorders. Therefore high levels of circulating visfatin are positively associated with cardiovascular disease, these may be due to localization of visfatin to foam cell macrophages within unstable atherosclerotic lesions, which presumptively play a role in plaque destabilization [23]. Oxidized LDL was verified to increase visfatin expression in cultured monocytes. Moreover, visfatin increased the expression of molecules that degrade extracellular matrix causing plaque instability. Also, visfatin can promote vascular smooth muscle inflammation, being associated with a potential role in vascular dysfunction and inflammation associated with some metabolic disorders [14].

Furthermore, we found GG genotype and G allele of the visfatin -948G/T to be associated with T2DM with CVD compared to T2DM without CVD (OR=3.56, P=0.034, OR=2.17, , P=0.012, respectively). Moreover, the frequencies of visfatin -948GG genotype and G allele were significantly increased in T2DM with CVD compared with T2DM without CVD. Our results were in agreement with results obtained by Bialy et al [17] who indicated higher frequencies of GG genotype and G allele than TT and T allele respectively. However, visfatin -948G/T variants was found to be associated with the apolipoprotein B component of VLDL and LDL levels.

Interestingly, -948G/T is in perfect or moderate linkage disequilibrium (LD) with other SNPs like -1001 T/G (rs9770242) and -423A/G (rs1319501) [16, 26]. Saddi-Rosa et al [26] suggested that the visfatin rs9770242 polymorphism associated with CAD in Brazilian subjects while no association was found in North-American subjects. A study in a Swedish cohort reported an association with myocardial infarction of the G allele of the rs1319501 a promoter SNP that is in complete linkage disequilibrium with rs9770242 and -948G/T [25]. Korner et al. genotyped 3 SNPs (rs9770242, -948G > T, rs4730153). The authors did not find association of any of the 3 polymorphisms or their haplotypes with glucose, insulin, or lipid levels [24].

Our observation that visfatin -948G/G polymorphism is a risk factor for the development of CVD in T2DM patients is supported by logistic regression we found that only TAG, TC, LDL-C, VLDL-C, TC/HDL-C, LDL-C/HDL-C and visfatin reliably predicted CVD.

Resistin is one of the most controversial adipocytokines which is a peptide hormone produced by adipocytes and macrophages [28], also known as FIZZ-3 (found in inflammatory zone 3) and participate in many metabolic pathways and inflammatory responses [43]. In recent reports, we found that serum resistin
level increased in T2DM compared with controls. So, resistin may be a key molecule for the development of insulin resistance; thus, serum resistin level could be a good marker to detect insulin resistance as well as T2DM. These results agreed with the results obtained by Uslu et al [33], Hivert et al [31], Osawa et al [32] and Tokuyama et al [34] suggesting that resistin plays an important role in the development of insulin resistance and T2DM among Turkish, Caucasian and Japanese populations respectively. On the contrary, Fujinami et al and McTernan et al who reported that the relation of resistin to glucose homeostasis and insulin resistance in T2DM is not consistent [29, 30].

Furthermore, our results revealed that significantly higher frequencies of the GG genotype and G allele of resistin −420 in T2DM patients than control group, the odds ratio (OR=4.86, \( P=0.033 \), OR=2.4, \( P=0.016 \) respectively) showed an association between the presence of the polymorphism and the prevalence of T2DM.

These results are in the same line with the results of previous studies El-Shal et al [28], Osawa et al [32] and Xu et al [46] who reported an association of resistin -420 with T2DM among Egyptian, Japanese and Chinese subjects, respectively. They also observed that significantly higher frequencies of GG genotype in T2DM patients than controls followed by CG and CC genotypes.

Moreover, Emamgholipour et al [44] investigated that the association between resistin polymorphism at -420C/G with T2DM in Iranian population; nonetheless, CC genotype compared to GG and CG increases susceptibility to T2DM and the CC genotype frequency was higher among the T2DM patients compared with healthy controls, although this difference was not significant. Ukkola et al [45] revealed that subjects with CC genotype showed higher fasting blood glucose, HbA1C and LDL levels in comparison to other genotypes. Also, they showed that the subjects with CC genotype had the highest insulin resistance.

On the contrary, in Thai population resistin -420C/G polymorphism was unlikely to play a major function in the etiology of T2DM [52]. In addition, Conneely et al [50] failed to detect any correlation between resistin polymorphism at -420C/G and T2DM among Finnish subjects. Ma et al [51] showed no significant association with T2DM was found at any of the polymorphic loci and resistin did not appear to be a major gene for T2DM. Meanwhile, meta-analyses by wen et al did not observe any association between the polymorphism of resistin -420 C/G and the risk of T2DM [53].

Our results revealed also, a significant increase in serum resistin level among T2DM with CVD compared to T2DM without CVD. In agreement with our result, Menzaghi et al [39] who showed that elevated serum resistin level is a risk factor for CVD in patients with T2DM of European ancestry. Lim et al [38] confirmed that resistin are proteins that affect insulin resistance and atherosclerosis significantly and investigated resistin concentrations as predictors of cardiovascular events in Korean patients with T2DM. In addition, Frankel et al [36] demonstrated that increased circulating concentrations of resistin were
associated with incident heart failure, even after accounting for prevalent coronary heart disease and measures of insulin resistance and inflammation. Norata et al [40] found that plasma resistin levels are associated with several metabolic and anthropometric parameters, and with cardiovascular risk in the general population.

Accordingly, in the review study by Jamaluddin et al [37] increasing resistin level indicated that it plays important regulatory roles a part from its role in insulin resistance and diabetes in a variety of biological processes: atherosclerosis and CVD. Furthermore, Uslu et al [33] suggested that increased serum resistin levels in T2DM may be novel biochemical risk factors for CVD complications. In addition, resistin has a pro-inflammatory role, which was shown to stimulate several factors, such as ET-1 (endothelin-1), VCAM-1 (vascular cell adhesion molecule-1) and MCP-1 (monocyte chemo attractant protein-1). Several studies have suggested resistin as a cardiovascular risk factor and examined its role in endothelial dysregulation and atherosclerotic lesion formation.

Our finding concerning an association between resistin -420 and CVD in T2DM. Meanwhile, the frequencies of resistin -420GG genotype and G allele were significantly increased in T2DM with CVD compared with T2DM without CVD. In accordance with our data, Hussain et al [47] who showed that there was a statistically significant difference in the resistin -420CG genotype distribution between HCM cases and controls and increased risk for cardiomyopathy in individuals with GG genotype and G allele compared with CC genotype and C allele, respectively among Pakistan population. Interestingly, Tang et al [49] who reported that the resistin-420C/G polymorphism might be associated with an increased risk of CAD in a Chinese population and the frequencies of -420C>G genotypes in the CAD group were significantly different from those in the control group. Subjects with the variant genotypes (CG and GG) had an increased risk of CAD compared to CC carriers.

Adding to these, Tsukahara et al [43] who suggested that the serum resistin levels were significantly high in patients with stroke and the genotyping of resistin polymorphism at -420C/G can be a risk marker for stroke susceptibility in Japanese T2DM patients. Also, individuals with the GG genotypes were significantly more likely to have had a stroke than individuals with the CC genotype. However, the frequencies of CC genotype and C allele were increased than GG genotype and G allele, respectively but they weren’t statistically significant.

Moreover, Ukkola et al [45] revealed that genetic variation had a role in the determination of plasma resistin level and -420CC genotype seemed to be associated with cardiovascular risk factors in Finnish population.

Furthermore, logistic regression supported our findings that resistin -420G/G polymorphism is a risk factor for the development of CVD in T2DM patients. We found that only TAG, TC, LDL-C, VLDL-C, TC/HDL-C, LDL-C/HDL-C and resistin reliably predicted CVD.
This agreed with, Miyamoto et al [48] who found that the G allele of the -420C/G SNP of the resistin gene increased susceptibility to metabolic syndrome, correlated with higher TAG and lower HDL-C. Osawa et al [32] plasma resistin, associated with SNP -420 and was correlated with low HDL cholesterol.

Surprisingly, we found that serum resistin was higher in GG carriers followed by CG then CC genotypes, this agreed with El-Shal et al [28], Tsukahara et al [43], Osawa et al [32] and Ukkola et al [45]. Thus, it is certain that the blood resistin levels are related to polymorphism at SNP -420C/G, which was supported by our study.

In conclusion, the results of our study supported the suggestion that visfatin -948G/T and resistin -420 C/G SNPs, as well as visfatin and resistin levels contributed to the development of T2DM and its CVD complications in Egyptian population. This conclusion is based on the serum visfatin and resistin levels were significantly high in patients with CVD in Egyptian type 2 diabetes. The prevalence of CVD was significantly correlated with the serum visfatin and resistin levels, which were increased according to the presence of visfatin -948G/T and resistin -420 C/G SNPs, respectively.

الملخص العربي

المرض السكري من النوع الثاني من الأضطرابات الأيضية حيث يتميز بارتفاع السكر في الدم واختلال مستوي الدهون والدهون البروتينية نتيجة إفراز إفراز الأديبوكين، أو مقاومة عمل الإنسولين أو كليهما معا. ومن المعروف أن مرض البول السكري يزيد من احتمالية الإصابة باعتلال الكلي، واعتلال الأعصاب، واعتلال الشبكية و أمراض القلب والأوعية الدموية، والتي تعد أكثر المضاعفات شيوعاً في المرضى المصابين بداء البول السكري.

وفي السنوات القليلة الماضية بزر إهتمام متزايد بدور الأديبوكين كعامل مباشر في الإصابة بداء البول السكري من النوع الثاني.

تم إفراز الأديبوكين عن طريق الخلايا الشحمية والخلايا الضامة المتسللة في النسيج الدهني مما يؤدي إلى حالة من الألتهاب الخفيف المزمن والتي يمكن أن تلعب دورًا في تطور مقاومة الإنسولين والأصابة بمرض البول السكري من النوع الثاني.

تم الحديث التعرض على الفسفاتين وهو أحد أنواع الأديبوكين التي تفرزها الدهون الحشوية ويلعب الفسفاتين دورًا مهمًا في تنظيم نسبة السكر في الدم. وقد تم أن هناك بعض أنواع الوراثات الجينية في جين الفسفاتين مرتبطة بالإصابة بداء البول السكري، وتم إثبات علاقة التورث الجيني الخاص بمخالطات الفسفاتين G/T. من العوامل السببية لداء البول السكري من النوع الثاني، ومضاعفاته كأمراض القلب العروضة.

وصف الريسستين في الأصل باعتباره هرمون خليقة شحمية محددة وقد اقترح ليكون حلقة وصل هامة بين السمنة ومقاومة الأنسولين وداء السكري. وكذلك فإن دراسة التغييرات الجينية في جين الريسستين أصبحت من أهم الأهداف لتحديد الاليات التي تشارك في الإصابة بمرض البول السكري من النوع الثاني.

لذا تهدف هذه الدراسة إلى تحديد الأشكال الجينية للفسفاتين والريسستين لدى المرضى المصريين المصابين بداء البول السكري النوع الثاني، وبعض مضاعفات وبعض مصاريع، ومقارنة ذلك بالأشكال الجينية لأشخاص طبيعيين غير مصابين بمرض البول السكري. بالإضافة إلى
ربط النتائج الخاصة بالتحاليل الجينية والتنميط الجيني بالبيانات الإكلينيكية وكذلك مستوي الفسفاتين والريسستين في مصل الدم بواسطة المعابرة الإنزيمية المرتبطة بالتمزاز المناعي.

ولقد أشتملت هذه الدراسة على 15 شخص (61 رجل و 89 امرأة) من المتردون علي مستشفى القصر العيني -جامعة القاهرة و تم تقسيمهم إلى: 90 مريضا بداء البول السكري من النوع الثاني و 60 شخص من الأصحاء متطابقين في الجنس والعمر.

وقد تم تقسيم المرضى المصابين بداء البول السكري من النوع الثاني إلى مجموعتين: المجموعة الأولى و تشمل 44 مريضا بداء البول السكري دون أمراض القلب والأوعية الدموية أما المجموعة الثانية فتشمل 44 مريضا بداء البول السكري ويعانون من أمراض القلب والأوعية الدموية.

وقد تم إجراء القياسات التالية:

1- حساب مؤشر كتلة الجسم.
2- قياس مستوي الجلوكوز (صائم) في الدم وكذلك مستوي الدهون الثلاثية والدهون البروتينية المنخفضة الكثافة والدهون البروتينية عالية الكثافة.
3- قياس مستوي الدهون الثلاثية في مصل الدم.
4- قياس مستوي الفسفاتين في مصل الدم.
5- قياس مستوي الريسستين في مصل الدم.
6- حساب معامل مقاومة الأنسولين.
7- حساب عوامل خطر أمراض القلب والأوعية الدموية 2.
8- حساب مستوي الدهون البروتينية المنخفضة جدا في الكثافة.
9- حساب مستوي الفسفاتين في الدم، مستوي الريسستين في الدم.

كما تم استخلاص الحمض النووي من الدم ثم تحديد النمط الجيني النووي الفردي عند نيوكليوتيد 948G/T. ونحو نيوكليوتيد 420C/G في جين الفسفاتين وعند هؤلاء المرضى المصابين بداء البول السكري من النوع الثاني حسب الدراسة.

وقد أظهرت نتائج هذه الدراسة ما يلي:

- حدوث اختلاف ذو دلالة إحصائية في نسبة السكر صائم، والدهون الثلاثية، والكوليسترول، والبروتين الدهني منخفض الكثافة في المرضى المصابين بداء البول السكري من النوع الثاني مقارنة بالمجموعة الضابطة، بينما لا يوجد اختلاف ذو دلالة إحصائية في مستويات هذه المعابير بين المرضى المصابين بداء البول السكري من النوع الثاني سواء يعتبران أو لا يعتبران من أمراض القلب والأوعية الدموية.

- حددت الدراسة أن نسبة السكر صائم، والدهون الثلاثية، والكوليسترول، والبروتين الدهني منخفض الكثافة في مصل الدم أعلى في المرضى المصابين بداء البول السكري من النوع الثاني، بينما منخفض مستوي البروتين الدهني عالي الكثافة في المرضى المصابين بداء البول السكري من النوع الثاني، بينما منخفض مستوي البروتين الدهني عالي الكثافة في المرضى المصابين بداء البول السكري من النوع الثاني، عن هؤلاء الذين يعتبران من داء البول السكري ولكن لا يعتبران من أمراض القلب والأوعية الدموية، إذ تم إجراء القياسات التالية.

- حدوث ارتفاع في مستوي الفسفاتين والريسستين في المرضى المصابين بداء البول السكري من النوع الثاني، عن هؤلاء الذين يعتبران من داء البول السكري ولكن لا يعتبران من أمراض القلب والأوعية الدموية، إذ تم إجراء القياسات التالية.
زيادة وجود النمط الجينيGG الخاص بجين الفسفاتين زيادة ذات دلالة إحصائية في مجموعة المرضى بداء البول السكري بدون أمراض القلب والأوعية الدموية إذا ما قورنت بمجموعة الأصحاء. أيضا زيادة تواجد النمط الجينيGG بجين الفسفاتين في المرضى المصابين بداء البول السكري من النوع الثاني ويعانون من أمراض القلب والأوعية الدموية عن هؤلاء الذين لا يعانون من أمراض القلب والأوعية الدموية زيادة ذات دلالة إحصائية بالإضافة إلى ارتفاع نسبة النمط الجينيGG في مرضى داء البول السكري من النوع الثاني ويعانون من أمراض القلب والأوعية الدموية.

وأرتفاع القلب عن غيرهم من يعانون فقط من داء البول السكري دون أمراض القلب والأوعية الدموية.

زيادة تواجد النمط الجينيGG بجين الريسستين في المرضى المصابين بداء البول السكري من النوع الثاني والذين يعانون من أمراض القلب والأوعية الدموية عن هؤلاء الذين لا يعانون من أمراض القلب والأوعية الدموية زيادة ذات دلالة إحصائية.

يرتبط الأختلاف النووي الفردي عند نيوكليوتيد-948G/T في جين الفسفاتين إرتباطا إيجابياً مع حدوث داء البول السكري من النوع الثاني حيث وجد ارتفاع في مستوى تركيزات الفسفاتين في مصل الدم لحاملين النمط الجينيGG مقارنة بأولئك الذين لديهم النمط الجينيGT وTT. بالإضافة إلى وجود ارتفاع في مستوى تركيزات الفسفاتين في مصل الدم لحاملين النمط الجينيGG في مجموعة المرضى بداء البول السكري من النوع الثاني وأمراض القلب والأوعية الدموية مقارنة بأولئك الذين لديهم النمط الجينيGT وTT مما يدل على ارتباط الأختلاف النووي الفردي عند نيوكليوتيد-948G/T في جين الفسفاتين إرتباطا إيجابياً مع حدوث داء البول السكري من النوع الثاني وكذلك مضاعفات أمراض القلب والأوعية الدموية.

الاختلاف النووي الفردي عند نيوكليوتيد-420C/G في جين الريسستين أظهر وجود ارتباط إيجابي مع حدوث كل من داء البول السكري من النوع الثاني إلى جانب مضاعفاته من أمراض القلب والأوعية الدموية، حيث وجد ارتفاع في مستوى تركيزات الريسستين CC في مجموعة المرضى المصابين بداء البول السكري من النوع الثاني ولا يعانون من أمراض القلب والأوعية الدموية بالإضافة إلى وجود ارتفاع في مستوى تركيزات الريسستين في مصل الدم لحاملين النمط الجينيGG مقارنة بأولئك الذين لديهم النمط الجينيCG وCC. من نتائج هذه الدراسة يمكن استنتاج ضعف التحور الجيني في كل من جين الفسفاتين وجين الريسستين إلى حدوث النمط الجينيGG التثير للأصابة بداء البول السكري من النوع الثاني ومضاعفاته من أمراض القلب والأوعية الدموية على الرغم من عدم تفسير كلا التحورين لزيادة وزن الصغير فقط من قابلية الأصابة بهذه الأمراض لذلك نتائجنا الحالية لإجراء المزيد من الأبحاث لتفعيل الأدلة الجزيئية في ضعف كل من جين الفسفاتين وجين الريسستين في قابلية الأصابة بداء البول السكري من النوع الثاني ومضاعفات أمراض القلب والأوعية الدموية.