Visfatin Level and Its Relation To Visfatin Gene SNPs -1001t/G and 1535 C/T – In Patients with Diabetic Nephropathy

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Abstract: Diabetic nephropathy (DN) is one of the most relevant diabetic complications. Visfatin is an adipocyte hormone expressed in peripheral blood neutrophils upon stimulation by inflammatory factors. Objective: to investigate the alteration of visfatin level and its relation to visfatin gene SNPs 1001T/G & 1535C/T in patients with DN. Subjects and methods: group I included 20 diabetic patients without nephropathy. Group II included 29 patients with DN& group III included23 subjectsas controls. They were subjected to history taking, BMI, FBG, fasting insulin, microalbumin, Hs-CRP, visfatin, and genetic analysis of 1535C/T &1001T/G SNPs by real-time PCR. Results: visfatin were higher in group I and group II compared with group III. There was a high significant difference between group I & II. Regarding SNP1001 T/G, the highest frequencies of TT genotype & T allel were found in group III (78.3% & 87.0%) followed by group I (55% &67.5%) and lastly group II (37.9% & 46.6%). Regarding TG/GG genotypes & G allel frequencies, the highest distributions were in the favor of group II (62.1% & 53.4%) then group I (45.0% & 32.5%) and finally group III (21.7% & 13.0%). There were statistical significant differences between the three studied groups regarding T & G alleles. By using odds ratio, TG/GG genotypes is more risky for DN 2.0times than TT &G allele is more risky for DN 2.38 times than T allele. Regarding 1535T/C SNP, no statistical significant differences were observe in genotypes and alleles in the studied groups. visfatinin individuals with the TG/GG genotypes in group II were higher than those with the TT genotype. Conclusion: visfatin level was significantly higher& correlated with microalbumin in patients with DN. Hence, it could be used as an early marker of renal endothelial dysfunction. SNP T/G 1001 may augment propensity to DN.
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Introduction:

Diabetes mellitus, especially type 2 (T2DM), represents one of the most important health problems worldwide [(1)]. According to data from the International Diabetes Federation, the number of diabetics older than twenty will rise from 285 million in 2010 to 439 million in 2030. Therefore, target organ complications secondary to diabetes, especially micro and macro vascular complications will be one of the most important medical concerns in the near future [(2)]Diabetic nephropathy (DN) is one of the most relevant diabetic complications. In the last decade, DN has become the main cause of end-stage renal disease[(3)].

Visfatin is an adipocyte hormone with a direct relationship between its level and T2DM[(4)]. Visfatin is a ubiquitous intracellular enzyme, also called nicotinamide phosphoribosyl transferase/pre-B cell colony-enhancing factor (PBEF)-1[(5)]. Besides expression in adipose tissue, visfatin is also expressed in peripheral blood neutrophils upon stimulation by inflammatory factors, such as tumor necrosis factor-α [(6)] .It has been reported that visfatin mimics actions of insulin by activating the insulin signal transduction pathway through binding to the same receptors. Therefore, it is implicated in the development of obesity associated insulin resistance and diabetes mellitus[(7&8)]. The visfatin/PBEF gene is located on chromosome 7q22.2 and consists of 11 exons and 10 introns, spanning 34·7kb of genomic DNA[(9)]. Visfatinwas found to have a role in progression ofdiabetic nephropathy[(10,11&12)]. Little records are obtained about the relation between visfatin gene polymorphisms and diabetic nephropathy.

Aim of the work:

The aim of the present study was performed to investigate the alteration of serum visfatin level and its relation to visfatin gene SNPs:1001 T/G &1535 C/T in TDM patients with diabetic nephropathy.

Subjects and methods:

The present study was carried out at Clinical Pathology Department, Faculty of Medicine, Menoufia University, in the duration between May 2012 and March 2014. The study was approved by Menoufiya University Ethics Committee and all subjects included in the study gave their written informed consent. The patients were selected from the Outpatient Clinics of Internal Medicine of Menoufia University Hospitals. The studied individuals were divided into 3 groups; group I consisted of 20 diabetic patients without nephropathy. They were 10 males and 10 females with ages ranged between 46- 63 years. Group II consisted

of 29 diabetic patients with nephropathy, 14 males and 15 females with ages ranged between 46- 62 years and group III whichconsisted of 23 apparently healthy subjectsserved as a control group. They were 12 males and 11 females with ages ranged between 45 – 60 years.

Exclusion criteria: patients with any kidney disease other than diabetic nephropathy, acute or chronic inflammatory or infectious disease, any psychiatric or neurological disorder, any malignancy, moderate to severe chronic obstructive lung disease, elevated liver enzymes & acute major cardiovascular events in the previous year.

All individuals were subjected to: Complete history taking, detailed physical 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).Laboratory investigation of fasting blood glucose, HbA1c, fasting insulin, lipid profile, kidney function tests, urinary microalbumin, serum visfatin, Hi-CRP &genetic analysis of SNPs 1001T/G& 15350 C/T of visfatin gene.

A. Sample collection and preparation:

1. Blood samples: Under complete aseptic conditions 6 ml of blood were collected by sterile venipuncture after 8 hours overnight fast and divided as follow: 1ml whole blood was collected in an eppendorf containing ethylene diamine tetra acetic acid (EDTA) 5% and stored at –20°C for genotyping of visfatin SNPs 1001T/G, 1535 C/T. 4 ml in plain vacutainer tube left to clot at 37ºC, sera were separated by centrifugation and then divided into two aliquots; one used for immediate assay of fasting blood glucose, lipid profile, kidney function tests and the other was kept frozen at -20 ºC for determination of visfatin, insulin & CRP till the time of assay. 1 ml was collected on EDTA tube for glycated haemoglobin (HbA1c).

2. Urine samples: Morning cleanly collected midstream samples were collected without preservative (10–20ml) for urine microalbumin and creatinine in urine.

B. Routine Laboratory Investigations:

They included estimation of serum glucose, creatinine, triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-C), microalbumin & creatinine in urine were done on SYNCHRON CX9 (Beckman, inst Inc,USA). Low-densitylipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. HbA1c by ion exchange chromatography (Stanbio Laboratory).

C. Specific laboratory tests:

1. Determination of serum visfatin using enzyme linked immunosorbant assay (ELISA)(Phoenix Pharmaceuticals, California, USA). Hs-CRP also using ELISA (Gen Way Biotech, Inc San Diego). Insulin was assayed by ELISA (DIA source INS-EASIA) for the homeostasis model assessment for insulin resistance (HOMA-IR). It was calculated as follow: HOMA-IR= fasting insulin uU/ml ×fasting blood glucose mg/ml divided by 405.

2. genetic analysis of SNPs 1001T/G& 1535 C/T of visfatin were studied by real time- PCR.

Genetic analysis:

DNA was purified from whole blood using Miniprep Kit for the purification of genomic DNA from whole blood (Axygen Prep Blood Genomic DNA). This method was based on the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and heme/protein precipitation buffer coupled with the selective adsorption of the genomic DNA to a special Axy Prep column. The purified genomic DNA was eluted in a low-salt Tris buffer containing 0.5 mM EDTA which enhanced DNA solubility and helped to protect the high molecular weight DNA against subsequent nuclease degradation. Following DNA isolation, SNPs (1001T/G &15350 C/T) of visfatin gene promoter region were studied by real time PCR for melting curve analysis (pplied Biosystem,7500 Fast Real Time PCR System, UK). We used 20 µl of reaction mixture containing 4µl DNA sample, 0.5 µl of each primer (Applied Biosystem, Life technology, UK), 0.2 µlM of each probe (Applied Biosystem, Life technology, UK), 10 ul of2x QuantiTect Probe PCR master mix (Applied Biosystem, Life technology, UK) in addition to 4.6 ul of RNase free water. PCR conditions and melting curve reading parameters were optimized. In the melting curve analysis, the polymorphic sequences demonstrated different melting peaks, representing a distinguishable melting point (Tm). The heterozygotes had both melting peaks. The primer and probesequences are shown in Table A.

Statistical analysis:

The statistical analysis was undertaken using SPSS software (version 17; SPSS Inc., Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviation for parametric data were used. Chi-square test ($\chi 2$) was used for qualitative variables. Man-witteny test for comparison between two groups having quantitative variables not normally distributed, ANOVA test for comparison between the three groups having quantitative variables normally distributed followed by LSD (least significant difference) and Kruskal-Wallis test for comparison between three groups not normally distributed having quantitative variables. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression analysis. The significance level was set at 0.05 or less.

Results:

Table A: Visfatin gene SNP primer and probe sequences.

1001T/G
Forward: 5'-GATAATGAGGGGACAAGACCTAA-3' Reverse: 5'-TGGAATGGTCTGTATTTGGGTGA-3' Anchor: 5'-GCAACGGGCCAAGCCTTTGAC-FL (Fluorescein) Sensor: 5'-LC-GGTGCGACACTGACTTTTATC-PH (Phosphate)
1535 C/T
Forward: 5'-ACTGGAGGCATGGCTGAGA-3' Reverse: 5'-CCCTCTTGTTTCAAACCTCGT-3' Anchor: 5'-ACAATACAGGGCAAAGATCATGGAAGTG-FL Sensor: 5'-LC—AAGGTATCACCAAGCACTCACC-PH

Table 1: Statistical comparison between the studied groups as regards different parameters

Variables	**Group I N =20**	**Group II N =29**	**Group III N =23**	**ANOVA**	**P value**	**Post hoc test**
BMI (kg/m2) X ± SD	27.51±2.01	28.23±2.37	24.7±1.56	**20.4**	<0.001	0.22 **a** <0.001**b** <0.001 **c**
Systolic BP(mmHg) X ± SD	130.0±11.23	140.0±17.32	120.0±0.0	**16.45**	<0.001	0.008 **a** 0.01 **b** <0.001 **c**
Diastolic BP(mmHg) X ± SD	86.0±6.8	88.96±8.06	80.0 ±0.0	**13.36**	<0.001	0.10 **a** 0.003 **b** <0.001 **c**
FBG(mg/dl) X ± SD	124.0±28.24	124.44±16.35	85.34±5.40	**35.13**	<0.001	0.93 **a** <0.001 **b** <0.001 **c**
HbA1c(%) X ± SD	7.13±1.06	8.44±0.90	5.78±0.56	**59.10**	<0.001	<0.05 **a** <0.001 **b** <0.001 **c**
TG(mg/dl) X ± SD	138.5±43.5	151.07±30.36	97.21±10.63	**20.96**	<0.001	0.16 **a** <0.001 **b** <0.001 **c**
Cholesterol (mg/dl) X ± SD	180.8±15.73	197.41±11.96	165.86±11.80	**37.67**	<0.001	0.06 **a** 0.05 **b** <0.05 **c**
HDL-c mg/dl X ± SD Median	79.8±7,50	59.37±10.10	77.08±5.29	**47.94**	<0.001	<0.001 **a** 0.28 **b** <0.001 **c**
LDL-c mg/dl X ± SD	43.0±7.52	74.37±14.42	67.56±10.61	**44.92**	<0.001	<0.001 **a** <0.001 **b** 0.04 **c**
HOMA-IR X ± SD	3.7±1.01	3.91±1.06	1.84±0.39	**39.4**	<0.001	0.41**a** <0.001 **b** <0.001 **c**
Hs.CRP(µg/ml) X ± SD	0.32±0.07	8.28±1.26	0.21 ± 0.04	**826.19**	<0.001	<0.001 **a** 0.66 **b** <0.001 **c**
Microalbumin ug/ mgcreatinine X ± SD	13.90±6.39	477.34±650.12	6.65±2.72	**58.58**	<0.001	.002 <0.001 0.002
Visfatin (ng/ml) X ± SD	**158.9±30.56**	**523.96±165.34**	**8.75±4.02**	**62.66***	**<0.001**	**<0.001 a <0.001b <0.001c**

>0.05 --Non significant <0.05 --Significant<0.01 -moderately significant <0.001-Highly significant(a) = Comparison between group I and II (b) = Comparison between group I and III(c) = Comparison between group II&III.

Table 2: Pearson correlation between visfatin and other parameters in groupI and group II.

Variables	Group I **(n=20)**		Group II (n=29)	
	Correlation coefficient (r) Visfatin	**P value**	**Correlation coefficient (r) Visfati**	**P value**
Age	+ 0.42	0.23	- 0.009	0.98
Weight	+ 0.25	0.48	+ 0.63	**0.050**
Height	- 0.08	0.81	+ 0.62	0.056
BMI	+ 0.27	0.44	- 0.59	**0.04**
Systolic BP	+ 0.16	0.66	+ 0.26	0.46
Diastolic BP	+ 0.17	0.63	+ 0.61	0.06
FBG	- 0.32	0.36	- 0.44	0.20
TG	+ 0.11	0.76	+ 0.35	0.31
Cholesterol	+ 0.01	0.97	+ 0.25	0.48
HDL	- 0.33	0.35	+ 0.06	0.86
LDL	+ 0.29	0.42	+ 0.47	0.17
Creatinine	+ 0.17	0.64	- 0.07	0.84
Hs. CRP	- 0.28	0.43	+ 0.71	**0.05**
Hb A1c	+ 0.13	0.72	- 0.35	0.31
Microalbumin	+ 0.02	0.95	+ 0.44	**0.006**
HOMA-IR	+ 0.43	0.06	+ 0.44	**0.02**

Table 3:**Genetic analysis results of the studied groups:**

Variables	**Group I N =20**	**Group II N =29**	**Group III* N =23**	X^2	P value
Genotype					
1001 T/G				2.64 [F]	0.10[1]
TT#	11(55.0%)	11 (37.9%)	18 (78.3%)	8.46	0.003[2]
TG & GG	9(45.0%)	18 (62.1%)	5 (21.7%)	1.39	0.24[3]
1535 T/C				0.37 [F]	0.71[1]
TT#	15(75.0%)	21 (72.4%)	19(82.6%)	0.75	0.38[2]
TC& CC	5 (25.0%)	8 (27.6%)	4 (17.4%)	0.04	0.84[3]
Alleles	**N = 40**	**N = 58**	**N = 46**		
1001 T/G				4.71	0.03[1]
T #	27 (67.5%)	27 (46.6%)	40 (87.0%)	18.3	<0.001[2]
G	13 (32.5%)	31 (53.4%)	6 (13.0%)	4.20	0.04[3]
1535 T/C				0.78	0.38[1]
T#	33(82.5%)	45 (77.6%)	41(89.1%)	2.39	0.12[2]
C	7 (17.5%)	13 (22.4%)	5 (10.9%)	0.35	0.62[3]

F = Fisher's Exact test 1 = Comparing group 1 and group III2 = Comparing group II and group III
3 = Comparing group 1 and group II.

Table (4): Odds ratio of genotypes and alleles of 1001 T/G SNP:

variables	**Studied groups**		**X^2 (P value)**	**Odds ratio**	**95% CI**
	Group I* N =20	**Group II N =29**			
Genotype					
1001 T/G					
TT#	11 (55.0%)	11 (37.9%)	1.39	**2.0**	**0.63 – 6.36**
TG & GG	9 (45.0%)	18 (62.1%)	(0.24)		
Alleles	**N = 40**	**N = 58**			
1001 T/G					
T #	27 (67.5%)	27 (46.6%)	4.20	**2.38**	**1.03 – 5.52**
G	13 (32.5%)	31 (53.4%)	(0.04)		

*= reference group

Table 5: Statistical comparison between the anthropometric and biochemical indices in GII stratified by SNP1001G/T:

Variables	GII		Mann Whitney U	P value
	TT N = 11	TG/GG N = 18		
BMI (kg/m2) X ± SD	**29.67±2.47**	**28.45±3.07**	**1.60**	**0.11**
FBG mg/dl X ± SD	**124.82±13.68**	**121.94±19.25**	**0.63**	**0.53**
Hb A1c % X ± SD	**8.54±1.09**	**8.28±0.78**	**1.24**	**0.21**
TG mg/dl X ± SD	**147.55±38.79**	**153.06±24.23**	**0.70**	**0.49**
Cholesterol mg/dl X ± SD	**194.27±13.58**	**197.89±10.81**	**0.52**	**0.60**
HDL-c mg/dl X ± SD Median	**59.54±9.89**	**60.17±11.35**	**0.34**	**0.74**
LDL-c mg/dl X ± SD	**69.18±13.84**	**77.67±14.07**	**1.53**	**0.13**
HOMA-IR X ± SD	**2.95±0.87**	**4.49±0.67**	**3.54**	**<0.001**
Microalbumin ug/mgcreatinine X ± SD	**115.0±32.38**	**583.0±775.86**	**2.68**	**0.007**
Hs.CRP(µg/ml) X ± SD	**8.06±1.44**	**8.05±1.43**	**0.11**	**0.91**
Visfatin (ng/ml) X ± SD	**369.09±40.11**	**592.5±163.13**	**3.93**	**<0.001**

Discussion:

The obtained results in this study showed that, the mean levels of visfatinwere statistically higher in group I (158.9 ±30.9 ng/ml) and group II (523.0±156.3ng/ml) compared with group III (8.75±4.0ng/ml)(P<0.001 for both). Moreover, there was a high statistical significant difference between group I & II (P<0.001). **Yilmaz et al.**[13] published that visfatin levels were positively associated with the degree of albuminuria in T2DM. They suggested that the endothelial dysfunction in early diabetic nephropathy is associated with altered circulating levels of visfatin. **Kang et al .**[5] found that plasma visfatin levels were significantly increased in T2DM irrespective of the degree of microalbuminuria. **Song et al.**[12] found in their study that visfatin was synthesized in renal glomerular mesangial cells, upregulated by high glucose stimulation. In addition, they found that exogenous visfatin stimulation in renal cells upregulated the synthesis of profibrotic molecules, including transforming growth factor-β1, plasminogen activator inhibitor, and type I collagen. Given these findings, the pathophysiologic relevance of visfatin seems to be as a proinflammatoryadipokine regarding T2DM and other metabolic complications[7].

In contrast to our results, Demir and co-workers [14] found that visfatin level was increased indiabetic patients without nephropathy than those with diabetic nepheropathy. They explained their results by another experimental study[15], where visfatin was shown to activate endothelial nitric oxide synthase via mitogen-activated protein kinase and monocyte chemoattractant protein-1 and improve endothelial cell function, angiogenesis, and atherosclerosis. Thus, controversy exists for the role of visfatin in diabetic nephropathy. Whether, this phenomenon primarily arises from direct vascular defects or is secondary to the presence of visceral obesity and a deranged metabolic milieu that is characteristic of many patients with diabetes remains unclear[7].

The current study (table 2) showed that there were significant positive correlations betweenvisfatin and weight & BMI in group II**.** The obtained results were in accordance with results of **Berndt et al.**[16]**.** Similarly, **Belo and colleagues**[9] observed a positive correlation between visfatin and BMI in their obese children**.** Obesity triggers the release of adipokines such as leptin, resistin, and visfatin, and these can then be associated with the progression of diabetic nephropathy and other vascular complications. These adipokines,

which are also synthesized in the kidney, appear to have an important role in renal injury associated with insulin resistance. It was found that visfatin is not only a surrogate marker of systemic inflammation in patients with T2DM but also up-regulated in diabetic kidney through the uptake of glucose into renal cells, which leads to the activation of the intracellular insulin signaling pathway and pro-inflammatory mechanisms [5]. Contrary to our results, **Pagano et al.** [17] observed a lower visfatin level in their obese subjects compared to non obese. Whereas, **Korner et al.** [18]found no association with measures of obesity. The controversial findings on visfatin levels as a result of obesity and metabolic syndrome could be explained by the fact that itis ubiquitously expressed in many tissues and the different organ's contribution to circulating visfatin level still needs to be defined[7].

The present study (table2) showed that there were significant positive correlation between visfatin and systolic blood pressure in group II.These results agreed with **Kang et al.**[11]. Also, the obtained results showed a significant positive correlation between visfatin and fasting blood glucose& HOMA-IR. These results agreed with **Kang & Cha**[5] and in contrast to the results obtained by **Berndt et al.**[16]In a study done by **Revolloet al.**[19] showed that visfatin is an essential enzyme in NAD production (Nampt). It exists both in intra and extracellular environments. Mice heterozygous for mutations in the visfatin gene have glucose intolerance mainly due to insulin secretion deficiency. This insulin secretion defect can be corrected by administering nicotinamide mononucleotide (NMN), the product of visfatin on NAD biosynthesis. Since the pancreas has very low levels of intracellular visfatin, the author suggests that maintenance of high NMN circulating levels by extracellular visfatin would be critical for normal beta-cell function**.** A negative correlation of visfatin levels with beta-cell function was demonstrated by studying acute insulin secretion assessed by an intravenous glucose tolerance test [20]**.**

The current study (table 2) revealed the presence of a significant positive correlation between visfatin&HbA1c**.** These results were in agreement with the study of **Zhu et al.**[21]**&** in contrast to a study done by **El-Mesallamy et al.**[22]. This observation is paradoxical but could be partially explained by the complex biology of visfatin/Nampt and the fact that it is involved in various processes like inflammation and NAD biology in addition to insulin resistance and metabolism[7].

The present study (table2) found that there was a significant positive correlation between visfatin and Hs-CRP (P<0.001) in group II. These results agreed with **Oki & Co-Workers** [23] who demonstrated that serum levels of visfatin were independently correlated with CRP and IL-6 in 295 Japanese Americans. Visfatin appears to be an important mediator of inflammation. **Moschen et al.**[24] demonstrated that recombinant visfatin induced dose-dependent production of pro-inflammatory IL-1β, TNF-α, and IL-6 as well as anti-inflammatory cytokines like IL-10, and IL-1 receptor antagonist in human monocytes. There are several possible mechanisms by which chronic low-degree inflammation might be induced in diabetes and its complications. In a hyperglycemic condition, the concentration of advanced glycation end products increases. Advanced glycation end products have been shown to activate macrophages, increase oxidative stress, and upregulate the synthesis of interleukin-1, interleukin-6, and tumor necrosis factor, resulting in the production of CRP[25]. Another possibility is that increase in CRP concentrations is related to adipose tissue derived cytokines [26]. It is suggested that circulating visfatin may promote not only IL-6, but also CRP [27].

The present study showed thatthere were significant positive correlation of visfatin with creatinine and microalbumin.These results agreed with **Nosheen et al.** who studied the association of visfatin with chronic kidney disease in a cohort of patients with and without diabetes[28]. **Yilmaz et al.** [29] studied patients of all CKD stages from stage1 to 5, and they found a higher level of visfatin in stage 3-5 as compared to subjects with stage 1-2 and controls but no significant difference was observed between controls and stage 1-2 CKD subjects. **Dogru et al.**[30] found that visfatin concentrations were significantly elevated in patients with microalbuminuria compared with those without and microalbuminuria.

The present study (table2) showed that therewere significant positive correlation betweenvisfatin with triglyceride, cholesterol and LDL-c (P<0.05) but negativecorrelation with HDL-C (P<0.001).**Smith et al.**[31]reported that serum visfatin level correlated positively to HDL-C.Also, **Gürsoy et al.**[32] showed that triglycerides correlated with serum visfatin levels in hyperlipidemic females**.** On the other hand, Samsam-Shariat and Co-workers[33]did not find any correlations between visfatin and lipid profile in patients with metabolic syndrome.

The relation between visfatin and lipid profile may be explained in the light of the cytosolic function of visfatin as a nicotinamide phosphoribosly transferase, an enzyme involved in NAD biosynthesis, which plays an important role as energy and signal transducer[34]. As inhibition of cholesterol ester protein increases HDL-C level and decreases LDL-C levels, it was proposed that visfatin in cholesterol homeostasis to be via inhibition of cholesteryl ester transfer protein [7].

Although some studieshave examined the relation between visfatin genotype and diabetes and related

metabolic disorders, there have been few studies related to the association between diabetic nephropathyand visfatin genotype. In this study regarding 1001 T/G polymorphism, the highest frequencies of TT genotype & T allel were recorded in group III (78.3%, 87.0%) followed by group I(55%& 67.5%) and lastly group II (37.9% &46.6%). Regarding TG/GG genotypes & G allel frequencies, the highest distributions were in the favor of group II (62.1% &53.4%) then group I (45.0%&32.5%) and finally group III (21.7% & 13.0%). There were no statistical significant differences neither between group I& III nor groupI & II regarding TT&TG/GG genotypes. Meanwhile, there were statistical significant differences between group II &III regarding TT & TG/GG genotypes. Moreover, there were statistical significant differences between the three studied groups regarding T&G alleles. By using odds ratio of these genotypes and alleles, TG/GG genotypes is more risky for diabetic nephropathy 2.0times than TT with (CI: 0.63–6.36). At the same time, G allele is more risky for diabetic nephropathy 2.38 times than T allele (CI: 1.03 – 5.52). Consequently, it is logical to guess that polymorphism may be interconnected with the incidence of where the G allele which may be the vital risk factor for diabetic nephropathy.

Regarding 1535T/C polymorphism, no statistical significant differences were observed in different genotypes (CC,CT&TT) and alleles (C&T) of this polymorphism between the studied groups. The results of Demir and Co-workers[14] were in accordance with our results. Tokunaga et al. found there was no significant difference in the frequencies of SNPs 1535C>T in the visfatin gene between the diabetic and control groups. This indicates that polymorphism did not increase susceptibility to type 2 diabetes mellitus [35]. Jian et al., [36] studied three SNPs; one of these was 1535 C/T. They found no difference in T2DM, impaired glucose regulation and normal glucose tolerance.

The current study addressed the question of whether visfatin level is related to SNP T/G1001 polymorphism and diabetic nephropathy. The results (table 5) revealed that level of visfatin and HOMA-IR in individuals with the TG/GG genotypes in group II were significantly higher than those with the TT genotype . Meanwhile, no statistical significances were detected between TT&TG/GG genotypes regarding other studied parameters. In a study done by **Carrero et al.** [37] found that subjects with 1001 TT genotype had higher visfatin levels than those with 1001 TG /GG genotypes The reason for this discrepancy may be that their study groups were chronic kidney disease. Axelsson et al[38] found no difference in circulating visfatin levels between genotypes. Moreover, Demir et al.,[14] found no relation between BMI, insulin resistance, serum lipid levels for this SNP.

There are some limitations to this study. First, the study groups are small. Second, dietary, antidiabetic & hypocholestraemic drugs intake which was suggested to be related with serum visfatin level was not evaluated.

Conclusion:

The mean visfatin concentrations were found to be statistically higher in diabetics compared to control subjects. Also, visfatin levels were found to be higher patients with diabetic nephropathy compared to diabetic patients without nephropathy. These findings suggest that visfatin is up regulated in diabetic nephropathy and hence could be used as a marker of renal endothelial damage. Moreover, obtained results suggesting that visfatin gene polymorphisms T/G 1001 but not C/T 1535 may augment propensity to diabetic nephropathy. Further studies to assess these polymorphisms and their association with visfatin expression and end-organ failure which may present new strategies in management of diabetes and its obstacles.

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