Serum chemerin levels and chemerin rs17173608 genotypes in the susceptibility of diabetic nephropathy in Egyptian diabetic patients

Yomna Khaled[a], Laila Rashed[b]

[a]Department of Internal Medicine, Faculty of Medicine, Cairo University, [b]Department of Biochemistry, Faculty of Medicine, Cairo University

Correspondence to Yomna Khaled, MD, Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt
Zip Code: 11562; Fax: (202)23682030;
e-mail: yomnakhaled@yahoo.com

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Background
Chemerin, a newly discovered adipokine, highly expressed in obese and insulin resistant patients may provide a link between chronic inflammation and metabolic syndrome.

Aim
Was to evaluate serum chemerin levels in diabetic nephropathy patients and to evaluate the susceptibility between rs17173608 chemerin gene polymorphism and diabetic nephropathy risk in Egyptian patients.

Materials and methods
Study was conducted on 105 patients having type 2 diabetes and twenty adult healthy matched controls. Patients were divided into three groups according to urinary albumin excretion (UAE), macroalbuminuric (UAE>300mg/24h), microalbuminurua (30<UAE< 300mg/24h) and normoalbuminuric (UAE<30mg/24h).Serum chemerin levels were measured to all patients and controls by enzyme linked immunosorbent assay.Tetra-amplification refractory mutation system-PCR was performed to detect gene polymorphism.

Results
Serum chemerin level was significantly elevated in diabetic patients compared to controls. There is significant increase in serum chemerin levels among diabetic subgroups, significantly higher in diabetic patients with macroalbuminuria than in patients with microalbuminuria (P < 0.001) and normoalbuminuria (P = 0.0001). Also it shows highly significant elevation in diabetics with microalbuminuria than in normoalbuminuria (P = 0.0001).Our findings showed a significant association between GT genotypes (OR: 2.95,95% CI = 1.06 to 8.1; P = 0.03 and diabetic patients with macroalbuminuria. In the dominant effect of the G allele (comparison between TG+GG and TT), TG+ GG genotypes were associated with the risk of diabetic macroalbuminuria (OR: 2.8, 95%CI = 1.08to 7.5; P = 0.03). The G allele is dominant and increased the risk of diabetic macroalbuminuria as compared to the T allele (OR = 2.8, 95% CI = 1.01to7.1, P = 0.03).

Conclusion
Elevated serum chemerin could be marker of diabetic nephropathy and chemerin gene rs17173608 polymorphism is associated with susceptibility of diabetic nephropathy.

Keywords:
chemerin, diabetic nephropathy, rs17173608 polymorphism

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Introduction

Diabetic nephropathy is a major serious complication of uncontrolled diabetes mellitus. Renal dysfunction in diabetic nephropathy is characterized by glomerular dysfunction, which leads to increased urinary albumin excretion (UAE), and has been shown to be associated with insulin resistance in patients with type 2 diabetes [1].

Several mechanisms have been implicated in the pathogenesis and progression of diabetic nephropathy [2]. Previous studies found an association between the secretion of inflammatory cytokines and the development of diabetic nephropathy [3]. Many clinical options targeting the pathogenesis of diabetic nephropathy have been attempted to manage and prevent its progression but still remain unsatisfactory as the number of diabetic nephropathy patients are increasing [2].

Chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2), tazarotene-induced gene 2 protein, is a novel adipocytokine that regulates adipocyte development, immune function, metabolic function, and glucose metabolism [4].

In humans, the expression of chemerin is increased in obese patients. Positive associations have also been found between serum chemerin and a component of metabolic syndrome [5]. Chemerin is also expressed by cells of the innate immune system. Chemerin has its role in inflammation and metabolism. Therefore, chemerin may provide a link between obesity and inflammation as well as obesity-related disorders such as type 2 diabetes and its complication such as diabetic nephropathy [6].

However, to date, there is no clinical study on chemerin gene polymorphism and risk for diabetic nephropathy. Thus, the aim of our study was to evaluate serum chemerin levels in diabetic nephropathy patients and to evaluate for the first time the association between rs17173608 chemerin gene polymorphism and diabetic nephropathy risk in Egyptian patients.

Patients and methods

Our study was conducted on 105 patients having type 2 diabetes, between January 2010 and January 2014. Patients were diagnosed as type 2 diabetes according to the American Diabetes Association guidelines. Patients were divided into three groups based on UAE. The first group included 35 adult diabetic patients with macroalbuminuria (UAE: >300 mg/24 h). The second group included 35 diabetic patients with microalbuminuria (30<UAE<300 mg/24 h). The third group included 35 diabetic patients with normoalbuminuria (UAE: <30 mg/24 h). Twenty healthy adults of matched age and sex served as a control group. All patients gave their formal consent. The protocol was approved the Ethical Committee of the Faculty of Medicine Cairo University.

Exclusion criteria included all patients with type I diabetes, patients with other causes of nephropathy apart from diabetes, and patients having inflammatory disorders.

All patients enrolled in the study were subjected to full medical history and clinical examination. Laboratory investigations were carried out for all patients and controls, including fasting blood glucose and kidney function tests (serum creatinine and blood urea nitrogen). HbA1c levels were measured using high-performance liquid chromatography. UAE was measured using the radioimmunoassay method.

Chemerin levels in sera were measured using a commercially available enzyme-linked immunosorbent assay (ELISA Kit; Quantikin R and D System, USA) Minneapolis, MN (Minnesota) according to the manufacturer's protocol. Genotype was determined for all patients and controls. Genomic DNA was isolated from peripheral blood leukocyte using DNA extraction kit supplied by Qiagene (Dusseldorf, Germany), according to the manufacturer's instructions. Gene amplification was carried out with a tetra-amplification refractory mutation system PCR using the following primers:

Forward inner (G allele): 5'-ATTGCTATAGTCCA GTGCCCTTCG-3', reverse inner (T allele): 5'-CCAGTTCCCTCTGTCGGCTTAA-3', forward outer: 5'-GTCAGACCCATGCAGTT TTCAAAC-3', reverse outer: 5'-GAGTTCC TCTCTCAAGCATCAGGG-3'.

PCR amplification was carried out in 25 μl reaction mixture using 100 ng DNA, 0.5 μl dNTP 10 mmol/l, 0.75 μl $MgCl_2$ 50 mmol/l, 10 pmol/μl of each primer, 0.3 U Taq DNA polymerase 5 U/μl (Qiagene). The PCR cycling condition was as follows: an initial denaturation for 5 min at 95°C, followed by 30 amplification cycles each of 90 s, 30 s at 95°C, 30 s at 62°C, and 30 s at 72°C, and then a final step for 10 min at 72°C was performed. PCR products were detected on 2% agarose gel stained with ethidium bromide. The amplification gives products of 262 bp for G allele, 322 bp for T allele, and 549 bp for two outer primers (control band) [7].

Statistical analysis

Statistical package for the social sciences (SPSS, version 22; SPSS Inc., Chicago, Illinois, USA) was used for analysis of data. Data were summarized using mean and SD for quantitative variables and frequencies (number of cases) and relative frequencies (%) for categorical variables. Comparison between groups were made using the unpaired *t*-test when comparing two groups, and analysis of variance with multiple comparisons post-hoc test when comparing more than two groups [8]. Genotype and allele frequencies were compared between the disease and control groups using the x^2 tests. Odds ratio (OR) with 95% confidence intervals (CIs) was calculated. For comparing categorical data, the x^2 test was performed. An exact test was used instead when the expected frequency was less than 5. *P* values less than 0.05 were considered as statistically significant.

Results

The clinical characteristics of diabetic patients and controls are shown in Table 1.

Our study showed that serum chemerin level was significantly higher in diabetic patients with macroalbuminuria (mean ± SD: 402 ± 55.94) when compared with controls (mean ± SD: 98.05 ± 11.42) ($P < 0.001$). Moreover, a highly significant elevation

Table 1 Demographic characteristics of diabetic subgroups and controls

	Normoalbuminuric diabetic patients		Macroalbuminuric diabetic patients		Microalbuminuric diabetic patients		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	51.34	5.09	53.26	7.26	51.63	6.98	51.15	6.21
Duration	3.3	1.35	8.83	2.92	5.50	2.10	-	-
Urea	24.69	3.94	158.26	42.14	63.89	13.92	48.40	7.64
Creatinine	1.103	0.16	6.89	2.65	1.88	0.95	1.42	0.31
UAE	16.32	5.78	525.83	150.57	92.49	19.46	12.47	3.44
Serum chemerin	99.44	10.60	402.26	55.94	255.77	43.28	98.05	11.42

UAE, urinary albumin excretion.

was detected in serum chemerin levels in type II diabetic microalbuminuric patients (mean ± SD: 255.77 ± 43.28) compared with controls (mean ± SD: 98.05 ± 11.4, $P < 0.001$). However, no significant difference was found in serum chemerin levels between normoalbuminuric type II diabetic patients (mean ± SD: 99.44 ± 10.6) and controls (mean ± SD: 98.05 ± 11.42) ($P = 0.65$) (Table 2).

A highly significant difference in serum chemerin level was observed between all patient groups. Serum chemerin level was significantly elevated in macroalbuminuric patients (mean ± SD: 402 ± 55.94) compared with microalbuminuric patients (mean ± SD: 255.77 ± 43.28) ($P < 0.001$) and normoalbuminuric patients (mean ± SD: 99.44 ± 10.61) ($P = 0.0001$). In addition, a highly significant elevation was observed in serum chemerin in microalbuminuric patients (mean ± SD: 255.77 ± 43.28) when compared with normoalbuminuric patients (mean ± SD: 99.44 ± 10.61) ($P = 0.0001$) (Table 3).

The genotype and allele frequencies of the chemerin rs17173608 are shown in Tables 4 and 5. The TT genotype frequency was 70, 65.7, 48.6, and 40% in controls, diabetic normoalbuminuric patients, microalbuminuric patients, and macroalbuminuric nephropathy patients, respectively, whereas the GG genotype was 10, 5.7, 8.6, and 8.5% in the above-mentioned groups, respectively. The GT genotype was 20, 28, 42.9, and 51.4%, respectively. The G allele frequency was 20% in controls, 20% in normoalbuminuric patients, 30% in microalbuminuric patients, and 34.3% in macroalbuminuric patients.

Our results showed a significant difference in the distribution of GT genotype between diabetic macroalbuminuric and normoalbuminuric patients. GT genotype is statistically significant in macroalbuminuric patients when compared to TT genotype (OR = 2.95, 95% CI = 1.06–8.1, $P = 0.03$). In the dominant effect of the G allele (comparison between GG + TG vs. TT), GG + TG genotypes significantly increased the risk for diabetic macroalbuminuria (OR = 2.8, 95% CI = 1.08–7.5, $P = 0.03$). The G allele frequency in diabetic macroalbuminuric and normoalbuminuric patients was 34.4 and 20, respectively.

The G allele increased the risk for diabetic nephropathy (macroalbuminuria) (OR = 2.8, 95% CI = 1.01–7.1, $P = 0.03$) compared with the T allele [Table 4].

Discussion

Diabetic nephropathy is a serious microvascular complication of diabetes mellitus. It is the leading cause of end-stage renal disease worldwide [3]. Several mechanisms have been implicated in the development of diabetic nephropathy. The exact cause of diabetic nephropathy is unknown, but various postulated mechanisms are hyperglycemia (causing hyperfiltration and renal injury), advanced glycation products, and activation of cytokines [9].

Chemerin, a novel adipocytokine, has been recently found to have a potential role in obesity-induced insulin resistance and inflammation [10]. Elevated levels of serum chemerin were recently reported to be significantly higher in patients on chronic hemodialysis compared with controls [11]. Therefore, serum chemerin levels can be altered in diabetic nephropathy.

Thus, the aim of our study was to evaluate serum chemerin levels in diabetic nephropathy patients and to evaluate for the first time the association between rs17173608 chemerin gene polymorphism and diabetic nephropathy risk in Egyptian patients.

As microalbuminuria is the earliest stage of diabetic nephropathy, we classified our type II diabetic patients according to the level of UAE to detect if serum chemerin and chemerin genotypes are present at early stage so that measures can be taken to prevent disease progression.

The present study revealed that serum chemerin was significantly elevated in diabetic patients compared with controls. This concurs with the study by

El-Mesallamy *et al.* [12], who reported that patients with type 2 diabetes had significantly higher chemerin levels compared with nondiabetic individuals; Elsebai *et al.* [13] also reported the same finding. This can be explained as follows: chemerin induces insulin resistance in peripheral tissues and inhibits glucose uptake [14]. Another explanation is that chemerin may exhibit an opposite effect on adipocytes where it increases glucose uptake, and so it stimulates insulin sensitivity. The increase in serum chemerin is a compensatory mechanism in patients with insulin resistance [15]. However, other studies failed to find a significant difference in plasma chemerin levels between diabetic patients and healthy controls [11,16]. Yamamoto *et al.* [17], in a study of diabetic patients on dialysis, concluded that serum chemerin levels were lower in diabetic patients compared with nondiabetic individuals and found that serum chemerin was associated with survival advantage.

Table 2 Comparison of serum chemerin levels between diabetic patients and controls

Serum chemerin (ng/ml)				
Macroalbuminuric diabetic patients		Control		*P* value
Mean	SD	Mean	SD	
402.26	55.94	98.05	11.42	<0.001
255.77	43.28			<0.001
99.44	10.60			0.65

Table 3 Comparison between serum chemerin levels in different study subgroups

Serum chemerin (ng/ml)				
Macroalbuminuric diabetic patients		Normoalbuminuric diabetic patients		*P* value
Mean	SD	Mean	SD	
402.26	55.94	99.44	10.60	0.0001
255.77	43.28			0.0001

In subgroups of type II diabetic patients, we found that serum chemerin was highly elevated in diabetic macroalbuminuric patients compared with microalbuminuric and normoalbuminuric patients. These results are in agreement with the results of previous studies [13,18]. Our explanation is that this may be due to increased glomerular enlargement in later stages of diabetic nephropathy, leading to increased loss of renal function and more albumin excretion, which leads to impaired chemerin excretion and increase in its serum level. This finding is also in agreement with previous studies on other adipocytokines, implying that chemerin as well as other adipokine degradation can be impaired in late stage of diabetic nephropathy [19].

Moreover, our present study revealed a highly significant difference in serum chemerin level between diabetic microalbuminuric and diabetic normoalbuminuric patients. This is in agreement with the findings of Elsebai *et al.* [13]. Serum chemerin is a significant differentiating marker between the diabetic subgroups. However, this finding was contradictory to other studies, which failed to show a significant difference in serum chemerin levels between controls and diabetic patients with microalbuminuria and normoalbuminuria [5,18]. This can be due to optimal glycemic control of their diabetic patients. Our results showed that there was no significant difference in serum chemerin level between diabetic normoalbuminuric patients and healthy controls. These findings suggest that serum chemerin could be used as a marker for diabetic nephropathy.

Table 4 Genotypes and risk for diabetic nephropathy in diabetic patients

Chemerin rs17173608	Macroalbuminuric diabetic patients (count (%))	Normoalbuminuric diabetic patients (count (%))	*P* value	OR (95% CI)
GG	3 (8.5)	2 (5.7)	0.35	2.46 (0.365-16.618)
GT	18 (51.4)	10 (28)	0.037	2.95 (1.067-8.195)
TT	14 (40)	23 (65.7)		Reference
Allele G	24 (34.3)	14 (20)	0.03	2.8 (1.104-7.180)
Allele T	46 (65.7)	56 (80)		Reference
TG + GG	21 (60)	12 (34.3)	0.03	2.8 (1.087-7.598)

CI, confidence interval; OR, odds ratio.

Table 5 Genotypes and risk for diabetic nephropathy in diabetic patients

Chemerin rs17173608	Microalbuminuric diabetic patients (count (%))	Normoalbuminuric diabetic patients (count (%))	*P* value	OR (95% CI)
GG	3 (8.6)	2 (5.7)	0.4	2.02 (0.304-13.512)
GT	15 (42.9)	10 (28)	0.17	2.02 (0.734-5.608)
TT	17 (48.6)	23 (65.7)	-	-
Allele G	21 (30)	14 (20)	0.13	2.02 (0.807-5.103)
Allele T	49 (70)	56 (80)	-	-
TG + GG	18 (51.4)	12 (34.3)	0.14	2.02 (0.775-5.313)

CI, confidence interval; OR, odds ratio.

In this study, we present novel findings of an association between chemerin rs17173608 gene polymorphism and risk for diabetic nephropathy in Egyptian patients. The chemerin rs17173608 polymorphism increased the risk for diabetic nephropathy in our study population. The results showed that rs17173608 may specifically contribute to diabetic macroalbuminuria.

In view of our results, serum chemerin levels were highly elevated in all diabetic nephropathy groups. To date, there are no studies showing the impact of chemerin polymorphism on diabetic nephropathy risk. Chemerin rs17176308 gene polymorphism contributes to overweight and obesity risk in Iranian women [20]. Mussig *et al.* [21] also showed that chemerin rs17173608 and rs10278590 polymorphisms were associated with increased visceral fat mass in nonobese individuals, not with total adiposity. Hashemi *et al.* [7] have discovered a significant association between G allele and risk for metabolic syndrome. Our study demonstrated that the G allele increased the risk for macroalbuminuria in type II diabetic patients. Moreover, the GT genotypes showed increased risk for macroalbuminuria in type II diabetic patients; hence, chemerin gene can predict progression of diabetic nephropathy.

Chemerin is an adipokine that plays a role in adipocyte metabolism, glucose metabolism, and regulation of immunity [22]. Chemerin expression was found to be elevated in patients with obesity and correlated positively with metabolic syndrome components. Chemerin mapping on 7q36.1 encodes an inactive form, prochemerin, which is activated through the cleavage of the C-terminus by inflammatory and coagulation serine protease [23,24]. Chemerin has a role in inflammation and metabolism; hence, it can provide an exciting link between type 2 diabetes and its microvasculsar complications such as diabetic nephropathy.

Conclusion

Our study concluded that elevated serum chemerin could be a marker of diabetic nephropathy and that rs17173608 polymorphism in the chemerin gene is associated with susceptibility of diabetic nephropathy progression in Egyptian diabetic patients. Further studies on rs17173608 chemerin gene polymorphism may be needed in different populations.

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Conflicts of interest

There are no conflicts of interest.

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