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Original Article

The association of serum chemerin levels with retinopathy in patients with type 2 diabetes mellitus

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Abstract

Objective: Diabetic retinopathy (DR) is one of the microvascular complications of type 2 diabetes mellitus (T2DM). A reliable biomarker is needed for the early diagnosis of this complication. The aim of this study was to investigate the relationship between serum chemerin and DR in T2DM patients.

Methods: The present study included 57 patients with T2DM and 20 healthy individuals. The T2DM patients were divided into three groups: nonretinopathy, nonproliferative retinopathy and proliferative retinopathy. Biochemical parameters, anthropometric measurements and ophthalmological examination findings of the participants were recorded.

Results: There was no significant difference between the chemerin levels of the patients and the control group (0.53(0.19-6.86) µg/ml vs. 0.7(0.25-3.88) µg/ml p=0.08), respectively. There was no significant difference between the patients with retinopathy and the control group for chemerin levels (p=0.13).

Conclusion: The present study showed that there was no significant difference in serum chemerin levels between T2DM patients and the control group. The reason for this may be that serum chemerin levels are affected by many other factors. Further studies are needed to determine whether chemerin has an effect on pathogenesis.

Keywords: Chemerin, diabetic retinopathy, type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) is an endocrine disease characterized by hyperglycemia caused by insulin deficiency and/or resistance (1). Diabetic retinopathy (DR) is a serious microvascular complication of diabetes. This condition has the potential to worsen and lead to blindness (2,3). There are two types of DR: nonproliferative diabetic retinopathy (NPDR), in which proliferation does not start and proliferative diabetic retinopathy (PDR), in which proliferation starts, characterized by neovascularization (4).

The pathophysiology of DR has not yet been clarified. Oxidative stress, proinflammatory mediators and increased levels of vascular endothelial growth factor (VEGF) have been emphasized in the pathogenesis of the disease (5). It is wellknown that chemerin acts in inflammation, adipogenesis and glucose metabolism. This suggests a potential effect of chemerin on the pathogenesis of DR (6).

Chemerin is an adipokine known to be produced from adipose tissue, liver, kidney, fibroblasts and various epithelial cells. The molecule is secreted inactively in the form of prochemerin and then activated by serine proteases of inflammation and coagulation by cleavage of the C-terminus (7,8). Previous studies have indicated that chemerin increases the proliferation of endothelial cells as an angiogenic factor that may stimulate vascular tube formation (9,10). However, studies evaluating the relationship with retinopathy in the literature suggest varying results (8,11-13).

In the present study, we aimed to determine how chemerin levels change in T2DM patients without retinopathy and those with retinopathy.

MATERIALS AND METHODS

The study was conducted prospectively in the internal medicine and ophthalmology outpatient clinics of Hitit University Medical Faculty Hospital. Approval was obtained from the Hitit University ethics committee (2022-18). In accordance with the Declaration of Helsinki, written informed consent was obtained from the participants.

Study population

The study involved 57 patients with T2DM who were followed up in the internal medicine outpatient clinic and 20 healthy volunteers without any disease who were examined in the ophthalmology outpatient clinic. When we included 19 healthy volunteers and 19 patients in our study, the power analysis was 95%.

We excluded patients with any systemic disease other than DM and hypertension, those with type 1 DM, T2DM patients with diabetic foot or diabetic nephropathy (elevated urea and creatinine levels), those who were treated because of DR (laser photocoagulation, anti-VEGF treatment), patients with ischemic heart disease and/or peripheral artery disease, deteriorated liver function tests, patients with a history of previous intraocular surgery and patients with glaucoma or uveitis.

Laboratory analysis

Blood samples of the participants were collected by an internal medicine clinic between 9 am and 11 am after a fasting period for one night (at least 10 hours) (approximately 6 to 7 ml venous blood). Some of the blood was sent to the hospital laboratory. HbA1c and fasting plasma glucose parameters were analyzed in this tube. The blood sample collected into another tube (approximately 3 ml) was placed in EDTA tubes. Blood samples were centrifuged at 3500 rpm for 10 minutes and serum was separated. Serum samples were taken and placed into eppendorf tubes. The samples were kept at -80°C until analysis. Before the analysis, the kits and serum samples were kept at room temperature (+25°C) for 60 minutes. Chemerin levels in serum samples were analyzed by the sandwich enzyme-linked immunosorbent assay (ELISA) method. The Bt laboratory catalog no: E1435Hu (Bioassay Technology Laboratory, Shanghai, China) Human chemerin ELISA kit was used for chemerin analysis.

Ophthalmological examination

Detailed ophthalmological exams of all participants were conducted by the same ophthalmologist. Fundus fluorescein angiography (FFA) (TRC-NW8F plus, Topcon Inc., Japan) was performed on patients with signs of retinopathy on fundus examination. According to the FFA findings (criteria of the American Academy of Ophthalmology), those with retinopathy were divided into two groups: NPDR and PDR. The values obtained by an optical coherence tomography (OCT, Spectralis, Heidelberg Engineering) device for macular and choroidal thicknesses of the participants were recorded. Measurements of the central ring with a radius of 500 µm were recorded for central macular thickness (CMT), and subfoveal choroidal thickness (SFCT) was measured manually (T. by marking (from the thinnest part of the macula) from the subfoveal area to the choroidal border. The measurements taken from the right eyes of the participants were used in the study. The body mass index (BMI) was calculated as BMI= weight (kg)/height (m^2).

Statistical analysis

IBM SPSS (Version 22.0, SPSS Inc., IL, USA) was used for statistical analysis. The conformity of the values to a normal distribution was evaluated by Kolmogorov–Smirnov analysis. Independent samples t test was used to compare normally distributed data and Mann–Whitney U test analysis was used for non-normally distributed data. One-way ANOVA was used for multiple (three and more) comparisons of the normally distributed data, while the Kruskal–Wallis test was used for non-normally distributed data. Any p value less than 0.05 ($p<0.05$) was taken as statistically significant.

RESULTS

The study included 77 participants (57 patients with T2DM and 20 healthy individuals). Among those with DM, 20 patients did not present findings of retinopathy (NDR), 26 patients had NPDR, and 11 patients had PDR findings. Thirty-two (41.6%) were male, and 45 (58.4%) were female. The mean age of the participants was 59.35± 8.90 years. There was no difference between the DM and control groups in terms of age and sex distribution ($p=0.23$, $p=0.49$, respectively). Table 1 shows the comparison of DM patients and control groups (Table 1).

Table 1. Comparison of clinical and biochemical characteristics of DM and control groups

	DM (n=57)	Control (n=20)	P
Chemerin (ng/ml)	0.53(0.19-6.86)	0.7(0.25-3.88)	0.08[a]
CMT (µm)	299.12±64.9	274.15±17.8	0.20[a]
SFCT (µm)	303.47±64.0	294.25±71.7	0.63[b]
BMI (kg/m^2)	30.54±5.2	28.38±3.4	0.12[a]
HbA1c (%)	8.80±2.3	5.17±0.4	**<0.01[a]**
FPG (mg/dl)	187.42±85.1	98.95±10.3	**<0.01[a]**

Abbreviations: CMT: Central macular thickness, SFCT: Subfoveal choroidal thickness, BMI: Body mass index, FPG: Fasting plasma glucose, a: Mann–Whitney U test, b: Independent samples t test, bold: $p<0.05$.

Table 2 shows the comparison of NDR and NPDR patients as well as the control groups. Figure 1 shows the 95% CI (confidence interval) plots for the mean chemerin value of the groups.

Table 2. Comparison of clinical and biochemical characteristics of retinopathy types and control groups

	DM			Control (n=20)	p[a]
	NDR (n=20)	NPDR (n=26)	PDR (n=11)		
Chemerin (ng/ml)	0.5(0.3-6.86)	0.54(0.19-6.62)	1.13(0.21-2.91)	0.7(0.25-3.88)	0.13
CMT (µm)	265.55±27.42	309.35±74.65	336.00±64.95	274.15±17.82	**<0.01**
SFCT (µm)	307.65±69.63	303.85±55.63	295.00±76.74	294.25±71.69	0.97
BMI (kg/m²)	31.85±5.83	30.10±5.40	29.21±3.00	28.38±3.38	0.24
HbA1c (%)	8±1.69	9.78±2.71	7.95±0.98	5.17±0.35	**<0.01**
FPG (mg/dl)	166.65±52.82	218.92±105.25	150.72±50.82	98.95±10.33	**<0.01**

Abbreviations: CMT: Central macular thickness, SFCT: Subfoveal choroidal thickness, BMI: Body mass index, FPG: Fasting plasma glucose, NDR: No diabetic retinopathy, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, BMI: Body mass index, a: Kruskal–Wallis test, bold: p<0.05

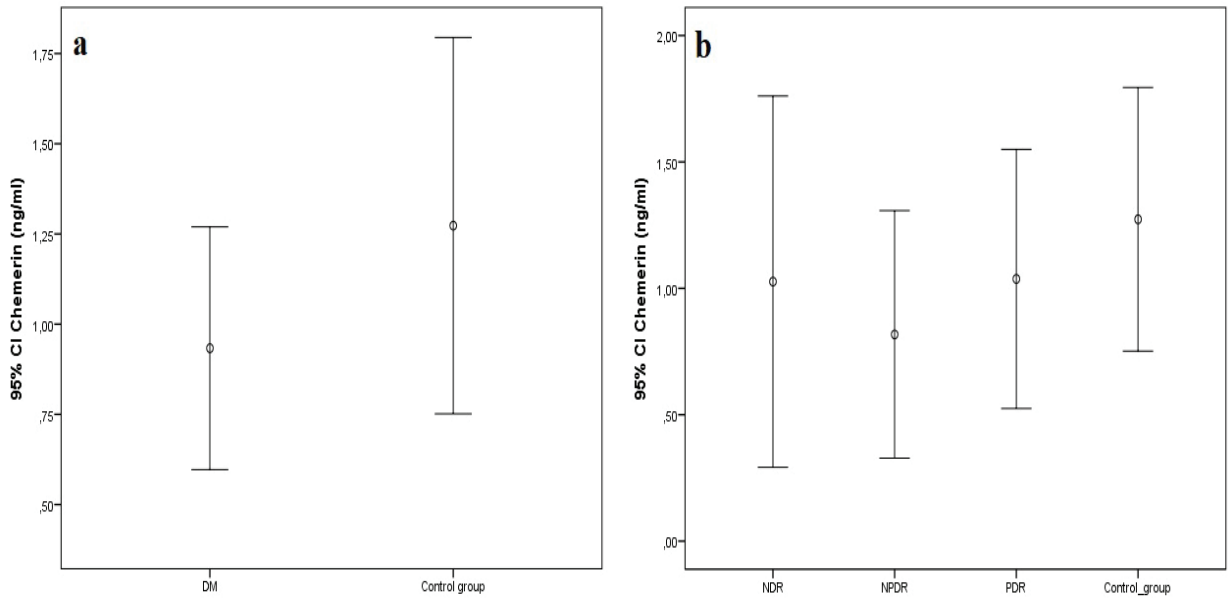

Figure 1.a) Comparison of chemerin levels in the DM and control groups. **b)** Comparison of chemerin levels in the retinopathy groups and control group.

In the correlation analysis, there was no significant correlation between chemerin levels and other parameters (Table 3). There was a strong correlation between HbA1c and fasting plasma glucose levels (r=0.820, p<0.001).

Table 3. Correlation analysis of chemerin and other parameters

	Chemerin (ng/ml)	
	r	p*
CMT (µm)	0.108	0.35
SFCT (µm)	0.024	0.84
BMI (kg/m²)	0.022	0.85
HbA1c (%)	-0.157	0.17
FPG (mg/dl)	-0.102	0.38

Abbreviations: CMT: Central macular thickness, SFCT: Subfoveal choroidal thickness, BMI: Body mass index, FPG: Fasting plasma glucose, r: Correlation coefficient *Spearman correlation test

DISCUSSION

DR is one of the causes of low vision in people of working age. Vascular endothelial dysfunction is very important in the pathogenesis of DR. Hyperglycemia, growth factors, cytokines and vasoactive molecules affect the functions of endothelial cells (14). Because chemerin, a recently discovered adipocytokine, is associated with inflammation, neovascularization and obesity, this molecule may also have an effect on the development of DR (6).

Li et al. showed that vitreous chemerin levels were elevated in patients with PDR compared to patients without DR. They also reported that increased VEGF levels increased inflammation in the vitreous and caused progression in DR and that VEGF and chemerin levels were correlated (15). Du et al. reported that chemerin serum concentrations were higher in patients with PDR than in patients with NPDR. However, there was no significant difference between chemerin levels in the group without retinopathy and the control group. This study reported that BMI, CRP and VEGF levels are associated with chemerin levels (6).

In their study, Bozaoğlu et al. found that serum chemerin levels were higher in T2DM patients. They found that chemerin levels were higher in the nondiabetic group, particularly in obese (BMI>30) individuals, than in normal (BMI<25) individuals (11). Tahir et al. found that the serum chemerin level was higher in the DM group than in the control group. They concluded in their study that higher chemerin levels may contribute to the pathogenesis of diabetic retinopathy by increasing inflammation, insulin resistance, oxidative stress and angiogenesis factors (12). However, in these studies, BMI of the DM group was higher than that of the control group. The reason why serum chemerin values were found to be higher in diabetic patients in these studies may be that BMI was higher in the diabetic group. In our study, there was no significant difference between the groups in terms of BMI values. This may be one of the reasons why chemerin values were similar between the groups. In support of this, some studies have shown that serum chemerin levels are affected by obesity-related factors (e.g., BMI, metabolic syndrome), insulin resistance, blood pressure, fasting plasma glucose, triglyceride levels and cholesterol levels (16-18).

No significant difference was found in serum chemerin levels between patients with DM and the control group in another group of studies (6,8,13). Halawa et al. and Du et al. found no significant difference in serum chemerin levels between the DM group and healthy individuals. When the groups with and without retinopathy were compared, the serum chemerin level was significantly higher in the retinopathy group. The authors reported in those studies that higher chemerin levels may have an effect on the pathogenesis of retinopathy by increasing inflammation, oxidative stress, and hyperlipidemia (6,13). Our study found no significant difference between the diabetic group and the control group or between the NDR, NPDR and PDR groups.

The reason why such different results were obtained in the studies was associated with the fact that chemerin acts as both an anti-inflammatory and pro-inflammatory molecule (19). Genetic factors may affect serum chemerin levels (20-24). Furthermore, the difference in nutrition has an effect on serum chemerin levels. A previous study on animals observed an increase in chemerin levels in mice fed a high-fat diet (7,25). In addition, in the results

obtained by ELISA in serum chemerin levels, active or inactive distinction of chemerin cannot be made. High chemerin levels in serum may not be directly related to chemerin bioactivity (26).

Limitations:

The limitations of our study were the lower number of patients (we had a hard time recruiting patients due to the COVID-19 pandemic), the lack of patients in the PDR group and the difference in diabetes duration in the patients. Another limitation was that lipid metabolism markers, C-reactive protein and similar markers were not included in our study. We believe that keeping BMIs and other variables that significantly affect serum chemerin levels similar between groups and increasing the number of patients would be beneficial in further studies.

CONCLUSION

In our study, there was no difference in serum chemerin levels between the DM group and the control group, or between the retinopathy groups and the control group. However, considering the different results in the literature, it seems that large-scale future studies are needed.

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References

1. Shi GJ, Shi GR, Zhou JY, Zhang WJ, Gao CY, Jiang YP et al. Involvement of growth factors in diabetes mellitus and its complications: a general review. Biomed Pharmacother. 2018;101:510-27.
2. Negi A, Vernon SA. An overview of the eye in diabetes. J R Soc Med. 2003;96:266-72.
3. Tielsch JM, Sommer A, Witt K, Katz J, Royall RM. Blindness and visual impairment in an American urban population: the Baltimore Eye Survey. Arch Ophthalmol. 1990;108:286-90.
4. Wilkinson CP, Ferris FL, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110:1677–82.
5. Pusparajah P, Lee LH, Abdul Kadir K. Molecular markers of diabetic retinopathy: potential screening tool of the future? Front Physiol. 2016;7:200.
6. Du J, Li R, Xu L, Ma R, Liu J, Cheng J, et al. Increased Serum Chemerin Levels in Diabetic Retinopathy of Type 2 Diabetic Patients. Curr Eye Res. 2016;41:114-20.
7. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem. 2007;282:28175-88.
8. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology. 2007;148:4687-94.
9. Kaur J, Adya R, Tan BK, Chen J, Randeva HS. Identification of chemerin receptor (ChemR23) in human endothelial cells: chemerin-induced endothelial angiogenesis. Biochem Biophys Res Commun. 2010;391:1762-68.
10. Landmesser U, Hornig B, Drexler H. Endothelial dysfunction in hypercholesterolemia: mechanisms, pathophysiological importance, and therapeutic interventions. Semin Thromb Hemost. 2000;26:529–37.
11. Bozaoglu K, Segal D, Shields KA, Cummings N, Curran JE, Comuzzie AG, et al. Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. J Clin Endocrinol Metab. 2009;94:3085-8.
12. Tahir NT, Falih IQ, Husaini FK, Zeghair SA. Study The Effect Of Chemerin Level In Type II Diabetic Patients With And Without Retinopathy. Systematic Reviews in Pharmacy. 2020;11:1856-63.
13. Halawa MR, Abdullah AA, Ibrahim NA, El-Sabawy AM. Chemerin is associated with diabetic retinopathy in type 2 diabetes. Egypt J Obes Diabetes Endocrinol. 2018;4:23-9.
14. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond). 2005;109:143-59.
15. Li J, Hu WC, Song H, Lin JN, Tang X. Increased vitreous chemerin levels are associated with proliferative diabetic retinopathy. Ophthalmologica. 2016;236:61-6.
16. Hart R, Greaves DR. Chemerin contributes to inflammation by promoting macrophage adhesion to

VCAM-1 and fibronectin through clustering of VLA-4 and VLA-5. J Immunol. 2010;185:3728–39.

17. Yang M, Yang G, Dong J, Liu Y, Zong H, Liu, H et al. Elevated plasma levels of chemerin in newly diagnosed type 2 diabetes mellitus with hypertension. J Investig Med. 2010;58:883–6.

18. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, et al. Insulin-sensitive obesity. Am J Physiol Endocrinol Metab. 2010;299:506–15.

19. Yoshimura T, Oppenheim JJ. Chemerin reveals ist chimeric nature. J Exp Med. 2008;205:2187-90.

20. Bozaoglu K, Curran JE, Stocker CJ, Zaibi MS, Segal D, Konstantopoulos N, et al. Chemerin, a novel adipokine in the regulation of angiogenesis. J Clin Endocrinol Metab. 2010;95:2476–85.

21. Er LK, Wu S, Hsu LA, Teng MS, Sun YC, Ko YL. Pleiotropic Associations of RARRES2 Gene Variants and Circulating Chemerin Levels: Potential Roles of Chemerin Involved in the Metabolic and Inflammation-Related Diseases. Mediators Inflamm. 2018;4670521.

22. Mussig K, Staiger H, Machicao F, Thamer C, Machann J, Schick F, et al. RARRES2, encoding the novel adipokine chemerin, is a genetic determinant of disproportionate regional body fat distribution: A comparative magnetic resonance imaging study. Metabolism. 2009;58:519–24.

23. Tonjes A, Scholz M, Breitfeld J, Marzi C, Grallert H, Gross A, et al. Genome wide meta-analysis highlights the role of genetic variation in RARRES2 in the regulation of circulating serum chemerin. PloS Genet. 2014;10:e1004854.

24. Wong TY, Klein R, Islam FM, Cotch MF, Folsom AR, Klein BE, et al. Diabetic retinopathy in a multiethnic cohort in the United States. Am J Ophthalmol. 2006;141:446-55.

25. Zhao L, Yamaguchi Y, Shen WJ, Morser J, Leung LLK. Dynamic and tissue-specific proteolytic processing of chemerin in obese mice. PloS One. 2018;13:e0202780.

26. Chang SS, Eisenberg D, Zhao L, Adams C, Leib R, Morser J, et al. Chemerin activation in human obesity. Obesity. 2016;24:1522–9.