Ann Med Res 2020;27(11):3086-92

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.01.042

Original Article

Association of Betatrophin, TNF-α and IL-6 with diabetic microvascular and macrovascular complications

Nevzat Gozel[1], Elif Melek[1] Burkay Yakar[2], Erhan Onalan[1], Mehmet Ferit Gursu[3]

[1]Department of Internal Medicine, Faculty of Medicine, Firat University, Elazig, Turkey
[2]Department of Family Medicine, Faculty of Medicine, Firat University, Elazig, Turkey
[3]Department of Biochemistry, Faculty of Medicine, Firat University, Elazig, Turkey

Abstract

Aim: The most important goal in the treatment of Diabetes Mellitus is to protect patients from micro and macro complications. Inflammatory cytokines and betatrophin have been reported to play a role in diabetic micro and macro complications. This study aimed to investigate the effect of serum Betatrophin, TNF-α and IL-6 levels on diabetic micro and macro complications in patients with type 2 Diabetes Mellitus.

Materials and Methods: This case-control study was conducted with 91 type 2 Diabetes Mellitus (T2DM) patients between January-December 2018. Case series with 91 patients divided into three groups were included; 31 T2DM patients without diabetic complications (group 1=control), 30 patients with diabetic microvascular complications (group 2) and 30 patients with diabetic macrovasculer complications (group 3). Blood was collected to evaluate biochemical parameters, TNF-α, IL-6 and betatrophin.

Results: Betatrophin level was high in patients with renitopathy (p=0.042) and diabetic foot (p=0.036). TNF-α levels were higher in patients with both microvascular and macrovascular complications compared to the control group (p<0.001). IL-6 levels were increased only in the group with macrovascular complications (p=0.027).

Conclusion: Betatrophin, TNF-α and IL-6 were associated with diabetic complications. Larger studies are warranted to establish the real impact of this finding.

Keywords: Betatrophin; diabetes mellitus; IL-6; TNF-α; Type 2/complications

INTRODUCTION

Diabetes mellitus is a common chronic disease characterized by insulin resistance and impaired insulin secretion with abnormal metabolic regulation of glucose metabolism (1). The incidence of diabetes mellitus is increasing day by day worldwide. According to the International Diabetes Federation (IDF), there were approximately 415 million people with diabetes in 2015 worldwide, and this number is expected to rise to 642 million by 2040 (2).

Diabetes mellitus have a sizeable impact on the global burden of morbidity and mortality on healthcare budgets both high prevalence and due to microvascular and macrovascular complications (3). Diabetes mellitus is currently the most important cause of preventable blindness and end-stage renal disease and is responsible for 40-60% of non-traumatic foot amputations. Diabetes mellitus increases 2-4 times the risk of cardiovascular disease which is the major cause of mortality (4).

The most important goal in the treatment of Diabetes mellitus is to protect patients from micro and macro complications of diabetes. In recent studies, inflammatory cytokines and betatrophin have been reported to play a role in diabetic micro and macro complications (5). Desislava et al. reported that serum IL-1, TNF-α and VEGF concentrations have an effect on the development and progression of diabetic retinopathy (6). Doganay et al. reported that increased serum sIL-2R, IL-8, TNF-α and NO levels in diabetic patients, with a significant correlation between the levels and the grade of diabetic retinopathy (7). Chen et al. reported that circulating betatrophin concentrations were significantly increased in type 2 diabetic patients with different stages of albuminuria, in particular macro albuminuric type 2 diabetic patients (8). In the literature, there are 4 different studies showing the relationship between betatrophin and diabatic retinopathy (9-12). Wang and et al. reported that serum betatrophin concentrations are increased in T2DM patients under antidiabetic treatment and positively associated with diabetic retinopathy (10).

Received: 12.01.2020 **Accepted:** 02.10.2020 **Available online:** 22.11.2020
Corresponding Author: Burkay Yakar, Department of Family Medicine, Faculty of Medicine, Firat University, Elazig, Turkey
E-mail: byakar@firat.edu.tr

Current research has focused on factors affecting diabetic macro and micro complications. The effect of cytokines and betatrophin on diabetic macro and micro complications is still controversial. The most effective way to protect patients from macro and micro complications of diabetes is by understanding the underlying mechanisms. This study aimed to investigate the effect of serum Betatrophin, TNF-α and IL-6 levels on diabetic micro and macro complications in patients with type 2 diabetes.

MATERIALS and METHODS

Study design and population

The study was conducted with 91 type 2 diabetes mellitus (T2DM) patients admitted to the endocrine outpatient clinic of tertiary university hospital between January-December 2018. In our study we included 31 T2DM patients without diabetic complications (group 1), 30 patients with diabetic microvascular complications (group 2) and 30 patients with diabetic macrovasculer complications (group 3). Patients followed up with the diagnosis of type 2 diabetes mellitus in the 30-70 age range were included in the study. Patients' exclusion criteria included: (1) Patients with additional diseases other than diabetes and complications, (2) Those who received antihyperlipidemic treatment such as statin, fibric acid, etc. (3) Mental retardation and psychiatric problems, (4) Current/recent infection (5) Malignancy. The study protocol was reviewed and approved by the scientific ethics committee of non-invasive researches of Firat University (date: 16.03.2017, number: 05-17). Written informed consent was obtained from all the enrolled patients prior to inclusion into the study in accordance with the Declaration of Helsinki.

Diagnosis of type 2 diabetes mellitus to all patients were performed by an endocrinologist according to the diagnostic criteria of American Diabetes Association. Patients with neuropathy, retinopathy or nephropathy included patients with diabetic microvascular complications. Patients with cardiovascular complications, periferic vascular disease, renal failure or cerebrovascular complications included patients with diabetic macrovascular complications. The presence of macrovascular or microvascular complications has been approved by the relevant specialist physicians.

Anthropometric and sociodemographic assessment

In our study, the anthropometric measurements of the participants were taken first. Height, weight and waist circumference of the participants were measured by the same researcher. BMI of participants were calculated with (BMI=kg/m2) formula after height and weight measurements. Demographic data of all participants such as age, gender, year of diabetes, family history of diabetes / gestational diabetes were recorded.

Biochemical investigations

From all patients, whole blood samples (total 5 ml) were obtained by veni puncture from a peripheral vein, avoiding haemolysis, into plain tubes; patients were in a resting position in the morning hours after an overnight fast. Fasting plasma glucose (FPG) levels as well as serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), calcium (Ca), phosphorous (P), chloride (Cl) and C-reactive protein (CRP)

were analyzed using an automatic biochemical analyzer (FUJI DRI-CHEM 4000i, Fuji,Japan). Hemoglobin A1c (HbA1c) concentration was measured using VARIANT II system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Serum levels of insulin were determined using UniCel DxI 800 Access Immunoassay System (Beckman Coulter, Inc., Brea, CA, USA).Fasting glucose and fastinginsulin levels were then used to estimate insulin

resistant state and β-cell function as follows: HOMA-IR (mg/dl)= [Fasting insulin (/mL) × Fasting glucose (mg/dl) / 405].

Measurement of plasma IL-6, TNF-α and Betatrophin levels

After 10-12 hours of fasting, 5 ml of additional venous blood from all patient groups were taken into aprotinin tubes for Betatrophin, TNF-α and IL-6.Plasma serum samples were separated and transferred to ependorf and stored at -80°C until working day. TNF-α and IL-6 concentrations were assayed by ELISA kits (YL biont, Shanghai, China) according to the manufacturer's protocols. The intra- and interassay coefficient of variation is <10 % and 12%, respectively. Betatrophin measurements were performed in the 3rd step hospital biochemistry laboratory by a commercial enzyme-linked immunosorbent assay (ELISA) (catalogue no. YLA2042HU; Shanghai YL Biotech Co.,Ltd., Shanghai. Assay range: 10 ng/L- 2000 ng/L, sensitivity: 5.21 ng/L, intra-assay CV < 8%; inter-assay CV < 10%).

Statistical analysis

Statistical analysis of the data was performed using IBM SPSS 22 statistical package program. Normal distribution of data was confirmed by the Shapiro-Wilk test before further analyses. Normally distributed data are expressed as mean±SD in the text and table and frequency for categorical variables as percentage [n (%)]. One-Way ANOVA test was used to compare more than two independent groups for normal distributed data. Post-Hoc Tukey test was used to compare the differences between the groups. Categorical variables were compared using Chi-squared and Fisher's exact tests, as applicable. A p-value of less than 0.05 was considered to be statistically significant for all analyses.

RESULTS

The mean age of 91 participants was 56.84 + 12.62 years and 53.8% (n=49) of the participants were female. The mean age of the participants in the group 3 (macrovasculer comp.) was higher than the mean age of the participants

in the other two groups (p<0.001). The duration of diabetes mellitus was found to be significantly higher in the groups that developed complications (p=0.021). The sociodemographic characteristics of the participants and the intergroup evaluation are presented in Table 1.

The mean fasting blood glucose levels of the participants with microvascular complications were higher than the other two groups (p=0.021). There was no statistical difference between the mean betatrophin levels of all three groups (p=0.271). The mean TNF-α levels of the participants with complications (group 2 and 3) were statistically higher than the group without complications (group 1) (p<0.001). IL-6 levels of the group with macrovascular complications were higher than the group 1 (p=0.007), but no statistical difference was found between the other groups (Table 2).

Table 1. Demographic and antropometric characteristics of patients

Variables	Group 1	Group 2	Group 3	p* value	p** value
Age					1-2:0.069
	50.38 ±12.33	55.4 ±11.37	64.93 ±9.65	**<0.001**	1-3:**<0.001**
					2-3:**0.002**
Gender					
Male	13	14	15	0.660	
Female	18	16	15		
					1-2:0.887
Height (cm)	169.43 ±10.39	161.10 ±8.33	163.97 ±8.38	0.404	1-3:0.279
					2-3:0.221
					1-2:0.801
Weight (kg)	77.72 ±14.69	78.73 ±15.52	83.05 ±16.45	0.368	1-3:0.185
					2-3:0.282
					1-2:0.806
BMI	29.93 ±6.57	33.3 ±5.43	31.33 ±6.69	0.226	1-3:0.385
					2-3:0.584
					1-2:0.327
Waist circumference (cm)	100.57 ±11.1	103.7 ±11.98	108.45 ±13.64	**0.046***	1-3:**0.014**
					2-3:0.135
					1-2:**0.014**
Duration of DM (year)	4.5±2.05	9.0±2.51	14.2±4.43	**0.021***	1-3:**<0.001**
					2-3:**0.007**
Smoking					
Yes	8	9	8	0.734	
No	23	21	22		
Alcohol use					
Yes	1	1	1	1.001	
No	30	29	29		
Family history of DM					
Yes	15	10	13	0.552	
No	16	20	17		

p*: multiple comparison p value.p**: binary comparison p value

Betatrophin levels in diabetic patients with microvascular complications were significantly different in diabetic retinopathy compared to uncomplicated diabetic patients (p = 0.042*) (Figure 1).

The relationship between betatrophin, TNF and IL-6 and diabetic macrovascular complications is shown in Figure 2.

Diabetic macrovascular complications were evaluated in subgroups, and the comparison within the group was statistically significant only in patients with diabetic foot problems (p=0.036*).

Table 2. Biochemical parameters of participants

Variables	Group 1	Group 2	Group 3	p* value	p** value
FBG (mg/dl)	130.30±37.20	185.63±59.05	151.87±68.71	**0.021**	1-2:**0.007** 1-3:0.464 2-3:**0.045**
PBG (mg/dl)	220.84±102.78	263.17±84.71	226.87±76.29	0.144	1-2:0.081 1-3:0.922 2-3:0.096
HbA1c (%)	6.66±1.51	8.24±2.49	7.48±1.54	**0.016**	1-2:**0.004** 1-3:0.154 2-3:0.128
Insulin	13.67±8.98	14.91±7.77	17.63±7.82	0.165	1-2:0.806 1-3:0.385 2-3:0.584
C-peptide	2.80±1.52	3.8±3.04	3.69±1.67	0.307	1-2:0.458 1-3:0.126 2-3:0.429
HOMA-IR (mg/dl)	4.32±2.86	7.83±5.33	7.90±5.77	0.074	1-2:0.051 1-3:0.126 2-3:0.429
Total Cholesterol (mg/dl)	199.43±42.79	201.37±40.46	184.13±47.87	0.284	1-2:0.872 1-3:0.201 2-3:0.149
Triglycerides (mg/dl)	180.93±58.15	205±67.04	166.93±56.64	0.106	1-2:0.164 1-3:0.489 2-3:**0.038**
LDL (mg/dl)	128.83±35.89	130.67±29.77	155.78±23.51	0.202	1-2:0.841 1-3:0.151 2-3:0.102
HDL (mg/dl)	48.61±9.42	46.62±6.89	42.68±10.08	0.603	1-2:0.565 1-3:0.318 2-3:0.674
AST	22.39±11.59	22.27±11.43	18.87±5.90	0.309	1-2:0.949 1-3:0.176 2-3:0.198
ALT	28.39±23.46	26±16.43	18.10±7.44	0.046	1-2:0.538 1-3:**0.017** 2-3:0.075
Total Protein (mg/dl)	7.01±0.70	7.26±0.67	7.10±0.53	0.231	1-2:0.089 1-3:0.334 2-3:0.447
Albumin (mg/dl)	4.19±0.55	4.62±0.49	4.90±0.50	0.101	1-2:0.656 1-3:0.111 2-3:0.052
Urea	32.55±18.53	45.13±9.79	66.50±4.84	**0.001**	1-2:0.571 1-3:**0.001** 2-3:**0.003**
Creatine	0.78±0.48	1.66±2.06	1.20±0.54	0.022	1-2:**0.008** 1-3:0.470 2-3:**0.047**
Hb	13.15±2.09	13.36±1.06	12.65±2.20	0.456	1-2:0.531 1-3:0.534 2-3:0.211
Htc	40.46±5.53	41.46±2.64	39.37±6.70	0.406	1-2:0.438 1-3:0.579 2-3:0.182
Plt	275.2±79.71	269.1±67.4	272.7±80.7	0.868	1-2:0.637 1-3:0.655 2-3:0.978
Urine density	1020.13±7.43	1022.60±10.19	1018.83±8.85	0.283	1-2:0.237 1-3:0.747 2-3:0.131
Betatrophin	928.87±90.41	963.54±98.77	949.74±110.95	0.271	1-2:0.124 1-3:0.232 2-3:0.718
TNF-α	313.78±74	395.74±44.51	355.64±70.24	**<0.001**	1-2:**<0.001** 1-3:**0.003** 2-3:**0.005**
IL-6	257.62±20.98	267.70±25.87	276.86±29.47	0.027	1-2:0.144 1-3:**0.007** 2-3:0.212

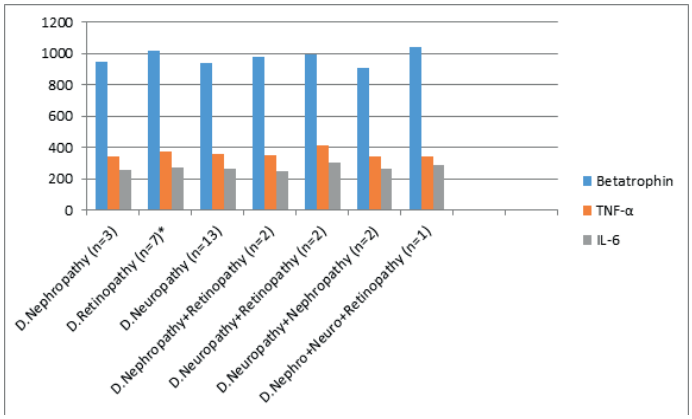

Figure 1. Relationship between microvascular complications and betatrophin, TNF and IL-6

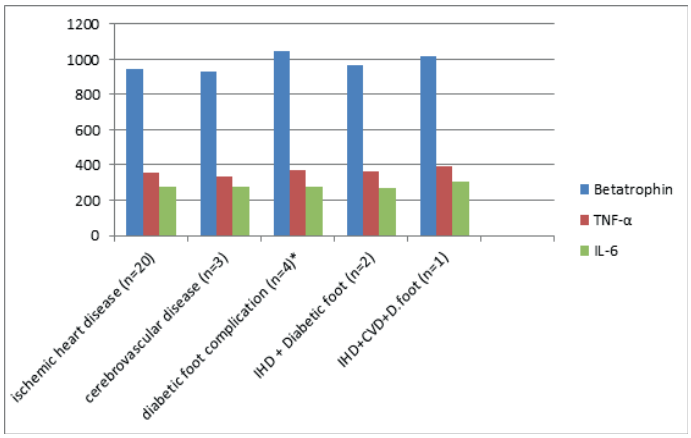

Figure 2. Relationship between macrovascular complications and betatrophin, TNF and IL-6

DISCUSSION

The present cross-sectional study focused on the relationship between T2DM complications and IL-6, TNF alfa and betatrophin levels. There were no differences in socioeconomic characteristics (such as gender, height, weight, BMI, smoking, and drinking alcohol) among in the three T2DM groups. However, the mean age of the group with macrovascular complications was higher than the other groups. The fact that we can not determine the mean age similarity among the groups is one of the limitations of our study.

In our study, there was no difference between the groups in terms of betatrophin levels. Betarophin is known to increase in diabetes mellitus (13). In our study, since all participants had type 2 diabetes mellitus, there might be no difference between the groups. In the literature, betatrophin levels were compared between diabetic and non-diabetic groups. Betatrophin has been reported to be elevated in patients with diabetes mellitus (13-15). In the literature, no similar study was found in which patients with diabetes were compared in our study. There are studies investigating the isolated effect of betatrophin with diabetic complications in the literature. In some studies, betatrophin has been reported to be associated with diabetic retinopathy and nephropathy (8,10,16,). In this study, although there was no difference between betatrophin levels between the groups but betatrophin levels were found to be higher in participants with diabetic retinopathy and diabetic foot complications.

There are several studies in the literature showing that betatrophin level is associated with diabetic retinopathy (9-12). Our data are consistent with the literature and betatrophin is thought to play a role in diabetic retinopathy. It can be investigated whether betarophine can be used as a marker for diabetic retinopathy in future researches.

In this study, betatrophin was found to be higher in patients with diabetic foot in the group with macrovascular complications. There were no studies in the literature about betatrophin and diabetic foot. To the best of our knowledge, this is the first study showing the relationship between diabetic foot and betatrophin. Although the relationship between diabetic foot and betatrophin was shown in our study, the small number of patients is the limitation of this study. We believe that our findings will lead to further studies with larger population in the future.

In 1998, a hypothesis was suggested that chronic inflammation leads to the development of type 2 diabetes mellitus (17). In the last few years, numerous studies have shown that low-grade inflammation is associated with the risk of developing type 2 diabetes mellitus (18). Furthermore, nowadays it is accepted that chronic subclinical inflammation is a part of the insulin resistance syndrome and is strongly related to features of the metabolic syndrome (19,20). Despite all these data, the mechanisms of chronic inflammation are unclear which can induce type 2 diabetes mellitus.

In the literature, in addition to inflammatory markers, inadequate diet is deemed to be a T2DM risk factor. There are reports regarding noteworthy associations of insulin resistance with high glycemic load diets (21). Recent studies have focused on the effect of TNF-α and IL-6 on diabetic complications. In this study both TNF alfa and IL-6 were higher in patients with diabetic complications. Many studies in the literature reported that TNF-α and IL-6 were higher in groups with diabetic complications (22-25). It is a widely accepted theory that TNF-α increases insulin resistance and leads to high blood sugar levels, thus leading to diabetic complications. In this study, TNF-α value was the highest in the group with microvascular complications. The mean fasting blood glucose and HbA1c levels were higher in the group with microvascular complications than the other groups. Our findings showed that TNF-α, fasting blood glucose and HbA1c are important in patients with microvascular complications. Although it is reported in the literature that TNF-α increases glucose by increasing insulin resistance so to causes diabetic complications we thought that it is unclear yet. The role of TNF-α in diabetic complications and its use as a marker should be investigated with extensive researches.

In the literature, IL-6, such as TNF-α and betatrophin, has been reported to increase in diabetes mellitus (25-27). In this study conducted among T2DM patients, IL-6 level was found to be higher in participants with macrovascular complications. To the best of our knowledge, there are no studies in the literature evaluating IL-6 level in T2DM patients. In limited studies investigating the relationship between IL-6 and diabetic complications, it has been reported that IL-6 plays a role in the etiology of diabetic nephropathy and retinopathy (28,29). Studies in the literature have focused on the relationship between IL-6 and diabetic microvascular complications. This study was conducted in a diabetic patients group with uncomplicated, microvascular complications and macrovascular complications. In the light of the literature, our expectation was high levels of IL-6 in patients with microvascular complications. Contrary to expectations, there was no difference between the group with microvascular complications and T2DM patients without complications. IL-6 levels of the patients with macrovascular complications were higher than those of the other two groups. IL-6 level is higher than other groups data is an unexpected and new finding. In the literature, the study investigating the relationship between IL 6 and diabetic complications is limited and to the best of our knowledge, there is no study comparing macrovascular complications and microvascular complications in diabetes mellitus. Our findings should be taken into consideration in future studies. Our data may change the focus of future studies and may provide a new perspective on the relationship between IL-6 and diabetic complications.

LIMITATION

The first limitation of our study was that the age factor could not provide homogeneity between the groups. Although we did not provide homogeneity in the age factor, the groups were similar in terms of other sociodemographic characteristics. Another limitation of our study was the cross-sectional and low number of patients. We can suggest that the findings obtained from our study should be investigated in larger populations.

CONCLUSION

In summary, although betatrofin levels did not differ between groups, we found that betatrophin level increased in patients with retinopathy and diabetic foot. We presented data to support the publications which are reported that betatrophin is increase in diabetic retinopathy. We present new data to the literature with our findings that betatrophin levels increase in patients with diabetic foot. We believe that this new data will provide a source for future studies. In this study, TNF levels were found to be high in patients with complications, especially in patients with microvascular complications. We thought that TNF level is found to be high especially in patients with microvascular complications may lead to new ideas. TNF levels were high in patients with diabetic foot complications. This data is a new finding for the literature and should be investigated with large population studies. IL-6 levels were higher in diabetic patients with macrovascular complications. This data has contributed to the literature as an unexpected and new finding. Furthermore extensive prospective longitudinal studies may analyze the role of betatrophin and proinflammatory cytokines in diabetic microvascular and macrovascular complications.

Conflict of interest: The authors declare that they have no competing interest.

Financial Disclosure: This study was supported by Firat University Scientific Research Projects Coordination Unit. Project Number: 2017:TF.17.15.

Ethical approval: The study protocol was reviewed and approved by the scientific ethics committee of non-invasive researches of Firat University (date: 16.03.2017, number: 05-17).

REFERENCES

1. Goldmann L, Schafer AI (editors). Goldman-Cecil Medicine. Textbook of Internal Medicine 2015;1150-2075
2. International Diabetes Federation. Annual Report 2015. https://www.idf.org/component/attachments.html?id=743&task=download access date 01.12.2019
3. 3. Alberti KG, Zimmet P. Global burden of disease--where does diabetes mellitus fit in? Nat Rev Endocrinol 2013;9:258-60.
4. International Diabetes Federation. IDF Diabetes Atlas, 7th ed. 2015. https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/13-diabetes-atlas-seventh-edition.html access date 01.12.2019.
5. Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1 beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. Folia Med (Plovdiv) 2011;53:44-50.
6. Desislava N, Georgieva K, Sivkova NP, et al. Serum inflammatory cytokines IL-1β, IL-6, TNF-α and VEGF have influence on the development of diabetic retinopathy. Folia Medica 2011;53:44-50.
7. Doganay S, Evereklioglu C, Er H, et al. Comparison of serum NO, TNF-alpha, IL-1beta, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. Eye (Lond) 2002;16:163-70.
8. Chen CC, Susanto H, Chuang WH, et al. Higher serum betatrophin level in type 2 diabetes subjects is associated with urinary albumin excretion and renal function. Cardiovasc Diabetol 2016;15:3.
9. Fang C, Huang Y, Guo H, et al. Lipasin, a biomarker of diabetic retinopathy. Diabetes Res Clin Pract 2016;115:96-8.
10. Wang YY, Zhang D, Jiang ZY, et al. Positive Association Between Betatrophin and Diabetic Retinopathy Risk in Type 2 Diabetes Patients. Horm Metab Res 2016;48:169-73.
11. Dong CX, Song CP, Zhang CP, et al. Clinical and experimental study on angiopoietin-like protein 8 associated with proliferative diabetic retinopathy. Int J Ophthalmol 2017;10:1819-23.

12. Lu Q, Lu L, Chen W, et al. Expression of angiopoietin-like protein 8 correlates with VEGF in patients with proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 2017;255:1515-23.
13. Li S, Liu D, Li L, et al. Circulating Betatrophin in Patients with Type 2 Diabetes: A Meta-Analysis. J Diabetes Res 2016;2016:6194750.
14. Espes D, Martinell M, Carlsson PO. Increased circulating betatrophin concentrations in patients with type 2 diabetes. Int J Endocrinol 2014;2014:6.
15. Ebert T, Kralisch S, Hoffmann A, et al. Circulating angiopoietin-like protein 8 is independently associated with fasting plasma glucose and type 2 diabetes mellitus. J Clin Endocrinol Metab 2014;99:257-10.
16. Liuxue Y, Jianfei S, Xiaoxi Z, et al. Association of Serum Angiopoietin-Like Protein 8 With Albuminuria in Type 2 Diabetic Patients: Results From the GDMD Study in China. Front. Endocrinol 2018;9:414.
17. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998;41:1241-8.
18. Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. Diabet Med 2004;21:203-7.
19. Festa A, D'Agostino R Jr, Howard G, et al. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42-7.
20. Fröhlich M, Imhof A, Berg G, et al. Association between C-reactive protein and features of the metabolic syndrome: a population-based study. Diabetes Care 2000;23:1835-9.
21. Villegas R, Liu S, Gao YT, et al. Prospective study of dietary carbohydrates, glycemic index, glycemic load, and incidence of type 2 diabetes mellitus in middle-aged Chinese women. Arch Intern Med 2007;167:2310-6.
22. Hernández-Da Mota SE, Soto-Bahena JJ, Viveros-Sandoval ME, et al. Pro-inflammatory serum cytokines in diabetic retinopathy. Cir Cir 2015;83:100-6.
23. George L. King. The role of inflammatory cytokines in diabetes and its complications. J Periodontol 2008;79(8 Suppl):1527-34.
24. Navarro JF, Mora C. Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. ScientificWorld Journal 2006;6:908-17.
25. Phosat C, Panprathip P, Chumpathat N, et al. Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural Thais: a cross-sectional study. BMC Endocr Disord 2017;17:44.
26. Wang X, Bao W, Liu J, et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2013;36:166-75.
27. Rehman K, Akash MSH, Liaqat A, et al. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. Crit Rev Eukaryot Gene Expr 2017;27:229-36.
28. Senthilkumar GP, Anithalekshmi MS, Yasir M, et al. Role of omentin 1 and IL-6 in type 2 diabetes mellitus patients with diabetic nephropathy. Diabetes Metab Syndr 2018;12:23-6.
29. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, et al. Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep 2017;7:2684.