The relationship of serum asprosin level with diabetic and non-diabetic retinopathy

H. ATLI[1], E. ONALAN[2], B. YAKAR[3], T. KAYMAZ[4], D. DUZENCI[5], K. KARAKULAK[2], E. DÖNDER[2], M.F. GÜRSU[4], R. DAYANAN[6]

[1]Department of Internal Medicine, Batman Training and Research Hospital, Batman, Turkey
[2]Department of Internal Medicine, Firat University, Medical School, Elazig, Turkey
[3]Department of Family Medicine, Firat University, Medical School, Elazig, Turkey
[4]Department of Bıochemıstry, Firat University, Medical School, Elazig, Turkey
[5]Department of Intensive Care, Inonu University, Medical School, Malatya, Turkey
[6]Department of Endocrinology, Batman Training and Research Hospital, Batman, Turkey

Abstract. – **OBJECTIVE:** This study was aimed at investigating the role of serum asprosin level in diabetic retinopathy pathogenesis and differential diagnosis diabetic and non-diabetic retinopathy.

PATIENTS AND METHODS: The cross-sectional study was conducted between May 2021 and August 2021. A total of 21 subjects with diabetic retinopathy, 21 subjects with non-diabetic retinopathy, 21 subjects with type 2 diabetes mellitus (T2DM) without retinopathy and 21 healthy controls were included in the study. Biochemical parameters, serum asprosin, serum IL-6 and TNF-α levels were measured in all participants.

RESULTS: Fasting blood glucose (FBG), HbA1c, HOMA-IR and LDL levels were higher in diabetic patients than non-diabetic. The blood asprosin levels were higher in the diabetic retinopathy group compared to the healthy control group (*p*=0.001), T2DM without diabetic retinopathy (*p*=0.010), and non-diabetic retinopathy group (*p*=0.043). There is a significant positive relationship between asprosin level and high FBG, HbA1c and HOMA-IR scores.

CONCLUSIONS: Serum asprosin level is significantly increased in DRP group than others. A high asprosin level might be a risk factor for the development of diabetic complications, such as diabetic retinopathy. These findings suggest that the measurement of serum asprosin level may support clinicians in determining the risk of DRP development.

Key Words:
   Diabetic retinopathy, Nondiabetic retinopathy, Asprosin.

Introduction

Diabetic retinopathy (DR) is one of the main reasons of vision loss as a result of uncontrolled blood glucose regulation and is one of the chronic complications of diabetes that eventually lead to blindness[1]. The only way to prevent vision loss due to diabetic retinopathy is to catch and treat patients in the prediabetes period and to keep their blood sugar under control and to follow them regularly. It is predicted that the number of patients with diabetic retinopathy in the United States will be 16.0 million in the coming years, and more than 3 million of them will suffer vision loss[2]. The benefit of tight glycemic control has been evident in large clinical trials that have been conducted[3,4].

Hyperglycemia causes the formation of glycation end products (AGEs) by activating various alternative glucose metabolism processes. Harmful substances resulting from these alternative routes cause vascular endothelial dysfunction and impairment of vascular permeability and microvascular occlusions by activation of cytokines. Retinal ischemia with microvascular occlusion leads to the formation of intraretinal microvascular abnormalities (IRMA) and neovascularization[5,6].

Although inflammation, apolipoprotein, vitamin D, oxidative stress and genetic factors are seen as factors in the etiopathogenesis of DR. It has been stated that many hormonal effects may play a role[7].

Asprosin is a hormone whose effect on glucose metabolism was recently discovered[8]. Asprosin is a protein consisting of 140 amino acids that is secreted from white adipose tissue in the event of hypoglycemia, stimulating gluconeogenesis from the liver and preventing hypoglycemia. The fact that Asprosin causes hyperglycemia and hyperinsulinemia in rat studies supports this hypothesis[8,9]. In a study[10], it was found that the increase in serum asprosin levels increased in proportion to glycemic indexes and insulin resistance (HOMA-IR).

Corresponding Author: Erhan Onalan, MD; e-mail: drakdeniz@msn.com

Considering this information, to the best of our knowledge, we are the first to study how asprosin levels in the blood of participants diagnosed with diabetic retinopathy, non-diabetic retinopathy and diabetes but without retinopathy are affected.

Patients and Methods

This cross-sectional study was performed in the Department of Endocrinology and Ophthalmology clinics between May 2021 and August 2021, in accordance with the Helsinki Declaration and after the Ethical Research Committee Approval (27.05.2021, IRB number: 07/04). Informed consents were obtained from all patients. We included total of 184 participants, including 21 patients diagnosed with Type 2 diabetes mellitus (T2DM) without retinopathy, 21 patients with diabetic retinopathy (DRP), 21 patients with non-diabetic retinopathy (non-DRP) and 21 healthy controls. Type 2 diabetes mellitus (T2DM) was diagnosed by an endocrinologist according to the American Diabetes Association guidelines. Participants aged 40-75 years old were defined as eligible for the study. T2DM was diagnosed based on either the fasting plasma glucose (FPG) of $\geq$126 mg/dL (7.0 mmol/L) or 2-h PG $\geq$200 mg/dL (11.1 mmol/L) during OGTT or glycated hemoglobin (HbA1c) $\geq$ 6.5% (48 mmol/mol), or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose $\geq$200 mg/dL (11.1 mmol/L)[11].

Diabetic retinopathy group was defined as patients with T2DM and retinopathy. Initially, patients were evaluated by the endocrinologist for the diagnosis of T2DM, and the diagnosis of T2DM was confirmed by the endocrinologist. The diagnosis of DRP was made by two ophthalmologists using an international scale[12]. Non-diabetic retinopathy groups defined as, patients with retinopathy and without diabetes mellitus. Firstly, the diagnosis of diabetes mellitus in patients was ruled out by the endocrinologist. Diagnosis of non-DRP was confirmed by two ophthalmologists taking into account absence of DRP diagnostic criteria. T2DM group was defined as patients with T2DM and without any retinopathy. The diagnosis of T2DM was confirmed by endocrinologist; in addition, two ophthalmologists confirmed no signs of retinopathy in patients. The control group consisted of healthy volunteers who did not report diabetes mellitus, retinopathy and any chronic disease. It was confirmed by the endocrinologist and ophthalmologist that the participants in the control group did not have diabetes mellitus and retinopathy.

Exclusion criteria included any other ocular diseases, systemic infection, malignancy, another type of diabetes than T2DM, steroid treatment and metabolic and endocrine diseases which can affect glucose metabolism.

Data Collection

Demographic characteristics and biochemical data of the participants were recorded. Anthropometric measurements of the participants were made and recorded by the same researcher. Body mass index (BMI) was calculated using the conventional Quetelet formula (BMI= kg/m^2). Overnight fasting three different venous blood samples (5 cc) were collected. Within 1 hour, a blood sample was sent to the clinical laboratory center of Firat University Hospital for further examination of the biochemical parameters. Biochemical parameters were measured using an automatic biochemical analyzer. Another blood sample was used for hemogram analysis. Five-milliliter blood (5 mL) sample was obtained for analyses of asprosin, TNF-α and IL-6. These samples were centrifuged at 5°C at 3,000 rpm and were stored at -20°C for further analyses.

Analyses of the Cytokine and Asprosin

Blood samples taken into tubes with aprotinin were centrifuged and stored at -20°C. Serum asprosin levels were measured using Enzyme-Linked Immunosorbent Test (ELISA) kits (Code: 201-12-9252) (Bioassay Technology Laboratory Shanghai, China). TNF-α and IL-6 concentrations were assayed by ELISA kits (YL biont, Shanghai, China) according to the manufacturer's protocols.

Statistical Analysis

IBM SPSS 22 (Armonk, NY, USA) statistical package program was used for this study. Shapiro-Wilk test was used for the distribution of continuous data. Descriptive data mean ± SD for normally distributed continuous variables, median (quartile 1-quartile 3) for non-normally distributed continuous data, and number (n) and percentage (%) for categorical variables. In the comparison of independent groups, One-Way Anova test was used for normally distributed continuous data, and Kruskal Wallis test was used for non-normally distributed continu-

Table I. Demographic, and laboratory data of the groups included in the study.

Variables	Control group (n=21)	T2DM without RP (n=21)	DRP (n=21)	Non-DRP (n=21)	*p*-value
Sex (female/male)	6/15	12/9	9/12	10/11	0.305
Age (years)	42.70±7.55	56.05±10.54	60.24±9.58	61.00±6.33	<0.001
BMI (kg/m²)	26.33±3.47	29.52±2.61	28.00±3.92	26.91±2.36	0.009
HbA1c (%)	5.19±0.61	9.51±3.32	9.83±2.48	5.54±0.42	<0.001
FBG (mg/dl)	87.00 (80.50-95.50)	177.00 (123.00-210.50)	175.00 (132.00-242.50)	90.00 (84.50-101.00)	<0.001
HOMA-IR	1.80 (0.94-2.21)	5.80 (2.54-10.24)	6.50 (3.99-19.17)	1.50 (1.15-2.50)	<0.001
T.Chol. (mg/dl)	188.24±30.50	192.57±36.76	189.67±53.85	169.05±44.75	0.265
LDL (mg/dl)	108.62±21.47	127.41±31.19	110.13±39.86	96.52±30.66	0.021
Triglyserit (mg/dl)	123.00 (88.50-171.50)	148.00 (113.50-190.50)	138.00 (105.50-262.00)	121.00 (91.50-164.00)	0.204
VLDL (mg/dl)	26.00 (17.60-42.50)	29.60 (23.00-39.50)	27.00 (21.00-60.00)	25.00 (17.00-37.00)	0.411
D-vit	23.40 (16.36-29.25)	17.40 (10.05-22.95)	12.40 (7.75-17.30)	16.20 (8.05-19.60)	0.008
AST (mg/dl)	19.00 (17.00-23.50)	19.00 (16.50-24.50)	16.00 (15.00-20.50)	25.00 (17.50-31.00)	0.021
ALT (mg/dl)	19.00 (14.00-29.50)	25.00 (16.00-32.00)	18.00 (14.00-26.50)	21.00 (15.00-32.00)	0.462
Hgb (g/dl)	14.90±1.13	13.71±1.78	13.08±1.79	13.04±2.18	0.003
PLT (*1000/µl)	238.62±43.01	268.82±25.79	275.38±84.87	227.38±60.43	0.129
BUN (mg/dl)	29.19±5.24	33.05±8.48	47.19±15.32	47.71±16.92	<0.001
Creatinine (mg/dl)	0.84±0.09	0.78±0.15	1.06±0.35	1.20±0.54	0.009
Insuline	7.20 (3.85-10.10)	12.10 (8.26-20.60)	22.70 (8.15-28.60)	7.00 (5.20-9.35)	<0.001
IL-6 (ng/L)	295.00 (264.50-410.00)	368.00 (274.00-415.50)	432.00 (295.50-557.50)	366.00 (335.50-473.50)	0.013
TNF-α (ng/ml)	1055.77 (801.99-1187.85)	1268.70 (909.08-1530.23)	1436.60 (1242.70-2244.00)	1807.57 (1512.30-3149.61)	<0.001
Asprosin (ng/ml)	25.49 (21.04-37.74)	28.22 (21.64-42.45)	57.45 (23.76-56.79)	38.49 (23.76-56.79)	0.005

FBG: fasting blood glucose.

ous data. Bonferroni test was used for post-hoc analysis. Statistical significance, *p*-value was <0.05.

Results

The characteristics of participants are shown in Table I. Compared with the participants with non-DRP and control group, the T2DM without RP and diabetic retinopathy groups had significantly higher BMI, HbA1c, glucose, HOMA-IR, LDL, triglyceride, platelet, insulin levels. Compared with the participants with T2DM and control group, the DRP and non-DRP groups had significantly higher urea, creatinine, IL-6 and TNF-α levels. Compared with DRP to non-DRP, diabetic retinopathy group had significantly higher HbA1c, glucose, HOMA-IR, triglycerides, platelet, IL-6 levels and significantly lower AST, and TNF-α levels than non-DRP group (Table I).

The levels of serum fasting asprosin level was also significantly higher in patients with diabetic retinopathy group than other groups

(*p*=0.005). The blood asprosin levels were higher in the diabetic retinopathy group compared to the healthy control group (*p*=0.001), T2DM without diabetic retinopathy (*p*=0.010), and non-diabetic retinopathy group (*p*=0.043). The blood asprosin level was not significantly difference between non-diabetic retinopathy, T2DM without retinopathy, and control groups (Figure 1).

We compared the serum fasting asprosin level with demographic and biochemical parameters between the groups (Table II). When the obtained data were analyzed, fasting asprosin level was positively correlated with creatinine, but negatively correlated with LDL and BUN levels in the control and T2DM groups without retinopathy. The fasting blood asprosin level was not associated with parameters of variables both in participants with diabetic retinopathy and non-diabetic retinopathy patients. When the correlation between asprosin level and other parameters is examined without dividing all data into groups, there is a significant positive relationship between asprosin level and FBG, HbA1c and HOMA-IR scores (Table II).
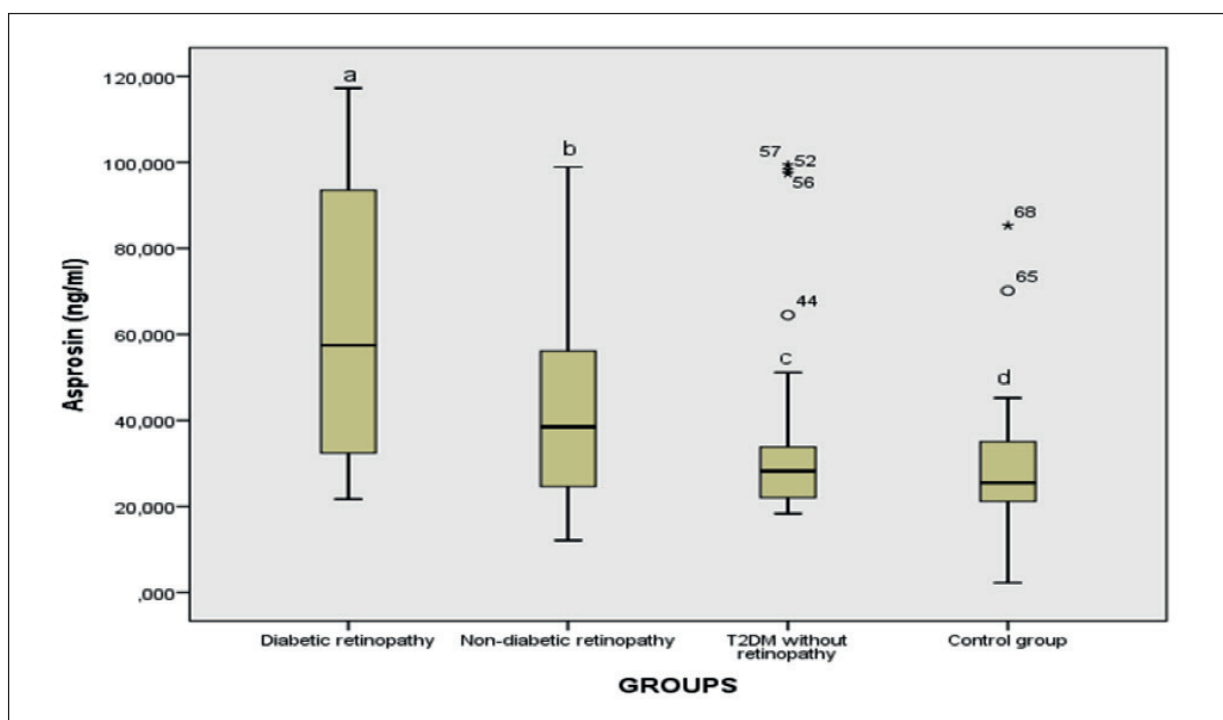
Figure 1. Asprosin level by groups (multiple comparison *p*-value=0.005, binary comparison *p*-value: a-b: 0.043; a-c: 0.010; a-d: 0.001; b-c: 0.552; b-d: 0.131; c-d: 0.355).

Discussion

It has been shown that asprosin can increase insulin resistance in skeletal muscle by reducing insulin signaling and increasing inflammation and endoplasmic reticulum stress. Recent scholars[13] have focused on the relationship between asprosin and hyperglycemia because of this effect. Current studies[14-16] reported that asprosin levels are associated with glucose and lipid metabolism, obesity and metabolic-related diseases and its concentration is higher in hyperglycemic patients compared to healthy individuals.

The current study showed that serum asprosin level is significantly higher in diabetic retinopathy than other three groups. On the other hand, there was no significant difference between the T2DM without retinopathy group and the non-DRP and control groups. Most studies[14,15,17-19] reported that asprosin level is higher in diabetic patients than control (non-diabetic healthy) groups. Consequently, it reflects the strong relationship between asprosin and hyperglycemia. Considering the above literature data, asprosin levels are expected to be higher in T2DM patients compared to the control group, but in the current study, no significant difference was found between the T2DM without ret-

inopathy group and control group. This study data contradicts previous studies. Gozel et al[18] reported that serum asprosin level increased in newly diagnosed T2DM and its level decreased significantly after metformin treatment. In this study, all T2DM patients have under antidiabetic treatment. Diabetes treatment may be the reason for the contradictory between the current study and the literature.

This study was focused on the relationship between serum asprosin and diabetic retinopathy and the role of asprosin in differential diagnosis between diabetic and non-diabetic retinopathy. The current study showed that serum asprosin level was positively associated with FBG, HOMA-IR and HbA1c levels. Wang et al[15] found that plasma asprosin concentration was positively correlated with FBG, PBG, HbA1c and HOMA-IR, similar to our study. The current study and previous literature data have shown that there is a relationship between increased asprosin level and high FBG, HbA1c and HOMA-IR scores. These findings suggested that there is a relationship between increased asprosin level and uncontrolled diabetes. It is known that diabetic complications are closely related with uncontrolled glucose levels. In the current study, we showed that serum asprosin level is associated with diabetic complication, such as retinopathy. In the literature,

Table II. Correlation analysis between possible affecting factors and asprosin concentrations by groups.

	Control		T2DM without RP		DRP		Non-DRP		Total (without dividing groups)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age (years)	0.048	0.842	0.127	0.583	-0.068	0.768	-0.128	0.581	0.202	0.067
BMI (kg/m²)	-0.010	0.965	0.022	0.924	0.016	0.946	-0.399	0.073	-0.125	0.257
FBG (mg/dl)	0.411	0.065	0.172	0.455	0.035	0.882	0.272	0.233	0.336	**0.002**
HOMA-IR	0.057	0.805	-0.100	0.668	0.058	0.803	0.299	0.187	0.246	**0.024**
HbA1c (%)	0.238	0.299	0.141	0.541	-0.034	0.885	-0.145	0.531	0.242	**0.027**
Insuline	0.016	0.944	-0.100	0.667	-0.134	0.561	0.282	0.215	0.176	0.109
T. Chol (mg/dl)	0.134	0.563	-0.262	0.252	0.100	0.666	-0.052	0.822	-0.109	0.322
Triglyceride (mg/dl)	0.041	0.859	0.215	0.350	-0.203	0.378	-0.228	0.320	0.045	0.686
LDL (mg/dl)	0.263	0.249	-0.454	**0.039**	0.010	0.966	-0.037	0.872	0.086	0.436
VLDL (mg/dl)	0.065	0.780	0.185	0.421	-0.077	0.740	-0.249	0.276	0.001	0.999
BUN (mg/dl)	-0.519	**0.016**	0.076	0.744	-0.170	0.461	-0.054	0.817	0.194	0.077
Creatinine (mg/dl)	0.471	**0.031**	0.156	0.500	-0.204	0.375	0.090	0.697	0.138	0.210
TNF-α (ng/ml)	0.112	0.628	0.035	0.880	0.393	0.078	0.007	0.977	0.198	0.071
IL-6 (ng/L)	-0.136	0.558	-0.237	0.301	0.101	0.662	-0.196	0.394	0.080	0.468
Hb (g/dl)	0.059	0.799	0.109	0.637	-0.256	0.263	-0.004	0.986	-0.214	0.051
Plt (*1000/µl)	-0.296	0.193	-0.120	0.603	0.271	0.235	-0.135	0.560	0.015	0.896

Total: correlation between asprosin level and other variables without groups.

two different studies[17,20] reported that elevated serum asprosin level is associated with diabetic nephropathy. Oruc et al[21] reported that serum asprosin level is higher in diabetic retinopathy patients than patients without DRP. The possible relationship between diabetic complications and serum asprosin level obtained in the current study is compatible with current literature data. Current study data and literature data suggest a relationship between diabetic complications and serum asprosin level.

Another hypothesis of the study was whether serum asprosin level could have a role in the differential diagnosis of DRP and non-DRP. The current study showed that serum asprosin level is higher in DRP group than non-DRP group. In the literature, there is no study on the role of asprosin in the differential diagnosis. Literature data showed that increased asprosin levels were associated with increased FBG, hbA1c and lipid levels. Based on these data, it was thought that there was a relationship between asprosin and uncontrolled diabetes, and as a result, it arranges the way for diabetic complications. This hypothesis is supported by the study showing that asprosin levels decrease in newly diagnosed diabetes patients who are treated. The fact that asprosin level was higher in patients with diabetic retinopathy compared to non-DRP patients in the current study suggested that there may be a relationship between asprosin and diabetic complications and that it can be used in the differential diagnosis. Although the obtained data support the relationship

between asprosin and diabetic complications, the literature data is insufficient to provide definitive evidence. A limited number of pathways were already unveiled although much more research is needed to better understand the therapeutic potential of asprosin in the diabetic complications.

Limitations

One of the limitations of this study is that it is single-center and the number of patients is small. Ignoring the stage of the disease in the diabetic retinopathy patient group is another limitation of the study. Factors that may affect diabetic complications (duration of illness, medication use, compliance with treatment, etc.) could not be standardized. Finally, different lifestyle factors and comorbidities relevant to the disease were not considered, which might potentially influence the results.

Conclusions

High asprosin level is associated with diabetic retinopathy and no relationship with non-diabetic retinopathy. A high asprosin level might be a risk factor for the development of diabetic complications such as diabetic retinopathy. Increased asprosin level may be due to hyperglycemia, insulin-resistant and worse diabetic complication such as diabetic retinopathy. Our data indicate that in

future studies, investigating the effect of asprosin-lowering treatments on diabetes complications, such as diabetic retinopathy, is needed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

A written informed consent was obtained from each patient. The study protocol was approved by the Ethics Committee of Fırat University (27.05.2021, IRB number: 07/04).

Patient Consent

Written informed consents were obtained from all patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

ORCID ID

Erhan Onalan: 0000-0001-5395-0390.

Authors' Contribution

HA: Data collection, design, Led and conceived the project, and authored the manuscript.
EO: Data collection, compiling, and discussion.
BY, TK: Contributed to collecting and analysis data, discussion.
DD, KK, RD: Contributed to collecting and analysis data.
ED, MFG: Contributed to collecting, statistics and analysis data.

References

1) Eisma JH, Dulle JE, Fort PE. Current knowledge on diabetic retinopathy from human donor tissues. World J Diabetes 2015; 6: 312-320.
2) Hendrick AM, Gibson MV, Kulshreshtha A. Diabetic Retinopathy. Prim Care 2015; 42: 451-464.
3) Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329: 977-986.
4) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998; 352: 837-853.
5) Behl T, Kotwani A. Exploring the various aspects of the pathological role of vascular endothelial growth factor (VEGF) in diabetic retinopathy. Pharmacol Res 2015; 99: 137-148.
6) Kador PF, Wyman M, Oates PJ. Aldose reductase, ocular diabetic complications and the development of topical Kinostat®. Prog Retin Eye Res 2016; 54: 1-29.
7) Matuszewski W, Stefanowicz-Rutkowska MM, Szychlińska M, Bandurska-Stankiewicz E. Differences in Risk Factors for Diabetic Retinopathy in Type 1 and Type 2 Diabetes Mellitus Patients in North-East Poland. Medicina (Kaunas) 2020; 56: 177.
8) Romere C, Duerrschmid C, Bournat J, Constable P, Jain M, Xia F, Saha PK, Del Solar M, Zhu B, York B, Sarkar P, Rendon DA, Gaber MW, LeMaire SA, Coselli JS, Milewicz DM, Sutton VR, Butte NF, Moore DD, Chopra AR. Asprosin, a Fasting-Induced Glucogenic Protein Hormone. Cell 2016; 165: 566-579.
9) Greenhill C. Liver: Asprosin - new hormone involved in hepatic glucose release. Nat Rev Endocrinol 2016; 12: 312.
10) Wang Y, Qu H, Xiong X, Qiu Y, Liao Y, Chen Y, Zheng Y, Zheng H. Plasma Asprosin Concentrations Are Increased in Individuals with Glucose Dysregulation and Correlated with Insulin Resistance and First-Phase Insulin Secretion. Mediators Inflamm 2018; 2018: 9471583.
11) International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009; 32: 1327-1334.
12) Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT; Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003; 110: 1677-1682.
13) Shabir K, Brown JE, Afzal I, Gharanei S, Weickert MO, Barber TM, Kyrou I, Randeva HS. Asprosin, a novel pleiotropic adipokine implicated in fasting and obesity-related cardio-metabolic disease: Comprehensive review of preclinical and clinical evidence. Cytokine Growth Factor Rev 2021; 60: 120-132.
14) Li X, Liao M, Shen R, Zhang L, Hu H, Wu J, Wang X, Qu H, Guo S, Long M, Zheng H. Plasma Asprosin Levels Are Associated with Glucose Metabolism, Lipid, and Sex Hormone Profiles in Females with Metabolic-Related Diseases. Mediators Inflamm 2018; 2018: 7375294.
15) Zhang X, Jiang H, Ma X, Wu H. Increased serum level and impaired response to glucose fluctuation of asprosin is associated with type 2 diabetes mellitus. J Diabetes Investig 2020; 11: 349-355.
16) Bhadel P, Shrestha S, Sapkota B, Li JY, Tao H. Asprosin and type 2 diabetes mellitus: a novel potential therapeutic implication. J Biol Regul Homeost Agents 2020; 34. doi: 10.23812/19-244-E. Online ahead of print.
17) Zhang H, Hu W, Zhang G. Circulating asprosin levels are increased in patients with type 2 diabetes and associated with early-stage diabetic kidney disease. Int Urol Nephrol 2020; 52: 1517-1522.
18) Gozel N, Kilinc F. Investigation of plasma asprosin and saliva levels in newly diagnosed type 2 diabetes mellitus patients treated with metformin. Endokrynol Pol 2021; 72: 37-43.

19) Zhang L, Chen C, Zhou N, Fu Y, Cheng X. Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triglyceride. Clin Chim Acta 2019; 489: 183-188.

20) Goodarzi G, Setayesh L, Fadaei R, Khamseh ME, Aliakbari F, Hosseini J, Moradi N. Circulating levels of asprosin and its association with insulin resistance and renal function in patients with type 2 diabetes mellitus and diabetic nephropathy. Mol Biol Rep 2021; 48: 5443-5450.

21) Oruc Y, Celik F, Ozgur G, Beyazyildiz E, Ugur K, Yardim M, Sahin I, Akkoc RF, Aydin S. Altered blood and aqueous humor levels of asprosin, 4-hydroxynonenal, and 8-hydroxy-deoxyguanosine in patients with diabetes mellitus and cataract with and without diabetic retinopathy. Retina 2020; 40: 2410-2416.