



Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Role of Fetuin-A and vascular endothelial growth factor In type 2 diabetes mellitus patients without and with retinopathy

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ARTICLE INFO

Article history:

Received 19 June 2019

Accepted 10 July 2019

1. Introduction

Diabetic retinopathy [DR] is due to long-standing diabetes, where chronic hyperglycemic in the body produces numerous complications [1]. But, in addition to hyperglycemia, other aspects, like hypertension, hemodynamic variations, abnormal lipid parameters etc., have a significant effect on the severity and clinical progression of diabetic retinopathy (DR) [2]. Visual loss progresses from increased permeability of retinal blood vessels or by the proliferation of new retinal vessels [3]. Frequently high glucose concentration in the body is found related to DR. But, the pathways that associate hyperglycemia and retinopathy are uncertain yet, and under investigation. Fetuin - A is secreted by the liver in adults. Fetuin-A is known to stimulate several potent angiogenic factors, including VEGF. Thus, it supports angiogenesis and indicates to neovascularization of blood vessels, which is one of the major pathologies of DR [4]. It is confirmed to an endogenous inhibitor of insulin receptor tyrosine kinase in the muscles and liver causing in decreased insulin signaling and insulin resistance [5]. Thus, Fetuin-A could be an early initiator of DR pathogenesis due to its dual role of insulin resistance and angiogenesis. Fetuin-A can, thus, be a marker of DR onset, as it participates both at the stage of insulin resistance as well as neovascularization. And also stimulate the proinflammatory action. Fetuin-A have been found associated with numerous pathological conditions including DM. Vascular endothelial growth factor (VEGF) is a signal glycoprotein that activates angiogenesis [6]. It is an effective pro-angiogenic factor, which

affects vascularization and endothelial function [7]. Increased expression of VEGF has an integral role in DR pathogenesis. VEGF is prominently involved in the pathogenesis of neo-vessels in diabetic retinopathy; leading to severe complication and irreversible loss of vision.

Oxidative stress (OS) results from either excess manufacture of free radicals or decreased effective concentration of antioxidants or both [8]. Oxidative stress is a potential cause for diseases such as the cardiovascular disease, Blood pressure related complications, Diabetes mellitus etc. [9]. Oxidative stress has been associated in the pathogenesis of DR. A variability of stimuli, like, hyperglycemia and hypoxia, might increase of production of ROS at the retinal level, causing oxidative stress [10]. Clinical studies propose that, with aging, in the presence of DR, oxidative stress levels expression a significant increase.

The present study focused on measuring the serum levels of Fetuin-A and VEGF, the molecules involved in insulin resistance, and angiogenesis. Oxidative stress was also assessed to correlate the function of the study molecules with free radical stress. This could provide information about the levels of these molecules in the serum, providing evidence to further understanding the currently unclear pathophysiology of DR in T2DM.

2. Subjects

The study comprised of two groups with 42 subjects in each group. Group A (controls) included type 2 diabetic patients without retinopathy and Group B (cases) included type 2 diabetic patients with retinopathy.

Inclusion criteria: Diabetes mellitus patients, diagnosed with DM-Type 2, without retinopathy, and with retinopathy. These

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patients included those taking oral hypoglycemic drugs; Age range: 35–70 years.

Exclusion criteria: Diabetic patients having comorbidities like infectious diseases, inflammatory conditions, hypo/hyperthyroidism and/or other known endocrinological disorders, morbid obesity, renal and liver impairment or presenting with psychological or neurological disorders. Women with gestational diabetes mellitus were also excluded.

3. Materials and methods

This is a Cross-sectional analytical study conducted in the Department of Biochemistry, JIPMER, with collaboration with the Departments of Ophthalmology, JIPMER, between the period of December 2017 and March 2019.

Sample size was estimated, with expected mean (\pm SD) differences level of Fetuin-A in patients without DR and with DR groups as 289 ± 30 μ g/mL and 310 ± 30 μ g/mL respectively, alpha error of 5% (95% confidence level) & power of 80%, we need 42 individuals in each group [11]. The sample size was calculated using OpenEpi software (version 3.0).

Ethical approval was obtained from the Institute Ethics Committee (JIP/IEC/2017/0341) and written informed consent was obtained from all the study subjects.

Baseline demographics and clinical data were collected after obtaining informed written consent from each study patients. Patients were recruited according to study criteria, and 5 ml blood was collected. The sample was obtained from the study subjects in fasting state. Serum was separated out from the collected blood, by centrifugation of the sample at 3000 rpm, for 5 min. This serum was used for the estimation of glucose and lipid profile, as well as, for the estimation of the special study parameters [Fetuin-A, VEGF]. The former was done using biochemistry auto analyzer. The latter was estimated Fetuin-A (Elabscience, USA), VEGF (Elabscience, USA), using ELISA kits purchased commercially and readings were taken using Biotech ELISA reader. Assessment of oxidative stress parameters, namely, MDA and TAS, were determined by spectrophotometric assays, by lipid peroxidation assay and FRAP assay, respectively.

TRIGLYCERIDES-GLUCOSE INDEX [TYG] is an indicator for insulin resistance [IR] [12].

$\text{TYG} = \text{Fasting triglyceride level (mg/dl)} \times \text{Fasting plasma glucose (FPG) (mg/dl)} / 2$

3.1. Statistical analysis

The data on baseline features, biochemical parameters and the outcome variables were expressed as Mean \pm Standard deviation. The comparisons of these variables between the groups were done by using Student's T test. Correlation between parameters was analyzed using Pearson correlation method. All statistical tests were two-tailed and p value < 0.05 was considered for statistical significance. Analyses were performed using SPSS version 20 software.

4. Results

4.1. Baseline demographic data of study subjects

The baseline characteristics compared between the study groups are given in Table 1.

Table 1

Comparison of baseline demographics between cases and controls.

Parameters	Cases (n = 42)	Controls (n = 42)	P value
Age (Years)	57.26 \pm 7.34	54.69 \pm 7.39	0.114
BMI (kg/m ²)	23.61 \pm 1.90	23.55 \pm 1.46	0.529
SBP (mm/Hg)	124 \pm 8.47	127.21 \pm 10.09	0.118
DBP (mm/Hg)	80.76 \pm 8.41	79.55 \pm 4.203	0.405

Data are Mean \pm Standard deviation. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

4.2. Biochemical parameters of study subjects

The comparison of clinical biochemistry parameters of the cases and controls are given in Table 2. Cases have significantly higher serum levels total cholesterol, LDL, and Triglycerides-Glucose ratio (TYG) in comparison to controls. Serum HDL level was significantly decreased in cases compared to controls.

4.3. Special serum parameters assayed between the study groups

Table 3: shows the results of comparison made between the major study parameters of the case and control groups. Fetuin-A, VEGF levels of cases are higher than that of controls, and also TAS levels decrease in cases, shows that statistically significant difference. The difference in MDA levels is not significant.

As shown in Fig. 1, significant positive correlations ($r = 0.249$, $p = 0.022$ (< 0.05)) were presented between fetuin-A and VEGF in the whole study population.

As shown in Fig. 2, significant positive correlations ($r = 0.243$, $p = 0.026$ (< 0.05)) were presented between TYG and Disease stages in the whole study population. Disease stages: 1-NDR, 2-Mild NPDR, 3-Moderate NPDR, 4-Severe NPDR, 5-PDR.

5. Discussion

Diabetic retinopathy (DR) is a vascular disease of the eye which disturbs patients with diabetes mellitus, and causing of reduced vision and loss of sight amongst adults [13]. Fetuin-A and VEGF are molecules participating the event of DR among many. There is evidence of insulin resistance and angiogenesis being a cause for the activity of these molecules in DR. Hence, we determined their circulating levels in T2DM, to investigate their relationship, along with oxidative stress in T2DM patients without and with retinopathy.

The study population showed almost equal status of BMI, between the cases and controls. This study involved subjects with an overall moderate risk [BMI \approx 23] for lifestyle complications, related to metabolic syndrome and Cardio vascular disease (CVD) risk, corresponding to the Asian population [14]. Assessment of lipid profile suggests that the patients "metabolic status" is toward a

Table 2

Comparison between biochemical parameters of the two groups.

Parameters	Cases (n = 42)	Controls (n = 42)	P value
FBG (mg/dL)	211.71 \pm 94.19	187.59 \pm 97.91	0.253
TC (mg/dL)	177.07 \pm 40.49	151.67 \pm 37.92	0.004*
TG (mg/dL)	159.29 \pm 66.20	144.67 \pm 94.01	0.412
HDL (mg/dL)	41.50 \pm 11.30	48.81 \pm 13.17	0.008*
LDL (mg/dL)	103.76 \pm 33.30	75.48 \pm 26.67	0.000**
TYG	5.12 \pm 0.27	4.97 \pm 0.41	0.044*

Data are Mean \pm Standard deviation.

*p value < 0.05 ; **p value < 0.001 considered statistically significant. FBG: Fasting Blood Glucose; TC: Total Cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein.

Table 3

Comparison between special parameters of the study groups.

Parameters	Cases (n = 42)	Controls (n = 42)	P value
Fetuin-A ($\mu\text{g/ml}$)	286.51 \pm 411.1	156.22 \pm 72.73	0.046*
VEGF (pg/ml)	947.11 \pm 797.86	642.55 \pm 505.93	0.040*
MDA ($\mu\text{m/L}$)	5.9 \pm 1.42	6.1 \pm 2.19	0.62
TAS ($\mu\text{m/L}$)	120.25 \pm 23.13	133.08 \pm 30.17	0.032*

Data are Mean \pm Standard deviation * p value < 0.05-statistically significant. VEGF: vascular endothelial growth factor; MDA: Malondialdehyde; TAS: Total antioxidant status.

more lipolytic approach, with circulating lipid components being elevated [15]. This study has observed significantly increased Total Cholesterol [TC], and LDL-Cholesterol levels in the cases. Although significant increase was not observed, the cases did show higher Triglyceride levels than the controls. We also observed a significant decrease in the HDL-Cholesterol levels of the cases, when compared to controls. Multiple population-based studies across the world have given evidences supporting a likely role of the atherogenic lipid profile in enhancing the severity of Diabetic Retinopathy. Results from the Early Treatment Diabetic Retinopathy Study (ETDRS), The Atherosclerosis Risk in Communities Study (ARICS), The Cardiovascular Health Study (CHS), and Hoorn Studies, have all converged at the likely association of serum Total Cholesterol and LDL-C with accumulation of retinal hard exudates in DR patients [16].

The current study observed no significant difference in the glycemic levels between the study groups. This could be because of the sample recruitment from subjects matched for the duration of their T2DM. However, a combined perspective of hyperglycemia and the atherogenic lipid profile was taken. The triglyceride-glucose index did show significant difference between the study groups. Hence, the effect of hyperglycemia is augmented by the

altered lipid profile, which could be associated to development and progression of DR [17]. The chronic hyperglycemic state may not be independently precipitating the diabetic complications. It is enhanced and sometimes dominated by the hand of lipid homeostasis and lifestyle-modifying factors. Previous studies have well documented significant positive correlation between the levels of Fetuin-A and the levels of atherogenic lipid profile [18,19]. Our study observed a significant increase in the Fetuin-A levels in the cases. However, we did not observe any significant correlation between the lipid profile and Fetuin-A, as with the previous studies.

But, the fact that a significantly elevated lipid profile exists in the DR disease condition points to the more unknown facets of DR pathogenesis, with respect to fat metabolism. In our study, we aimed to decipher where Fetuin-A stands in the pathogenesis of DR. Fetuin-A is a transporter of Free Fatty Acids. It has a suspected interplay with the lipoprotein components of lipid transport [20]. Previous studies have unraveled the role of Fetuin-A in altering adipose metabolism. It promotes lipolysis and efflux of the free fatty acids from the adipose into the circulation [21]. Thus, it probably increases circulatory plasma lipid concentration. We suspect the probable role of Fetuin-A in establishing disease, through its role in altering the lipid homeostasis, in DR. In addition, Fetuin-A knockout mice exhibited increased insulin sensitivity, supporting the hypothesis that Fetuin-A is involved in the pathophysiology of insulin resistance in rodents [5].

TyG, a novel index of insulin resistance was assessed in our study to determine the insulin resistance state [22]. Our study identified a significantly increased TyG index in patients with DR. It also positively correlated to the severity of the disease. It clearly put forth the fact that insulin resistance is an important mediator of DR progression. We have not observed any correlation between Tyg index and Fetuin-A. But, the progressive resistance to insulin could probably be an effect of increased Fetuin A levels. Fetuin-A alters

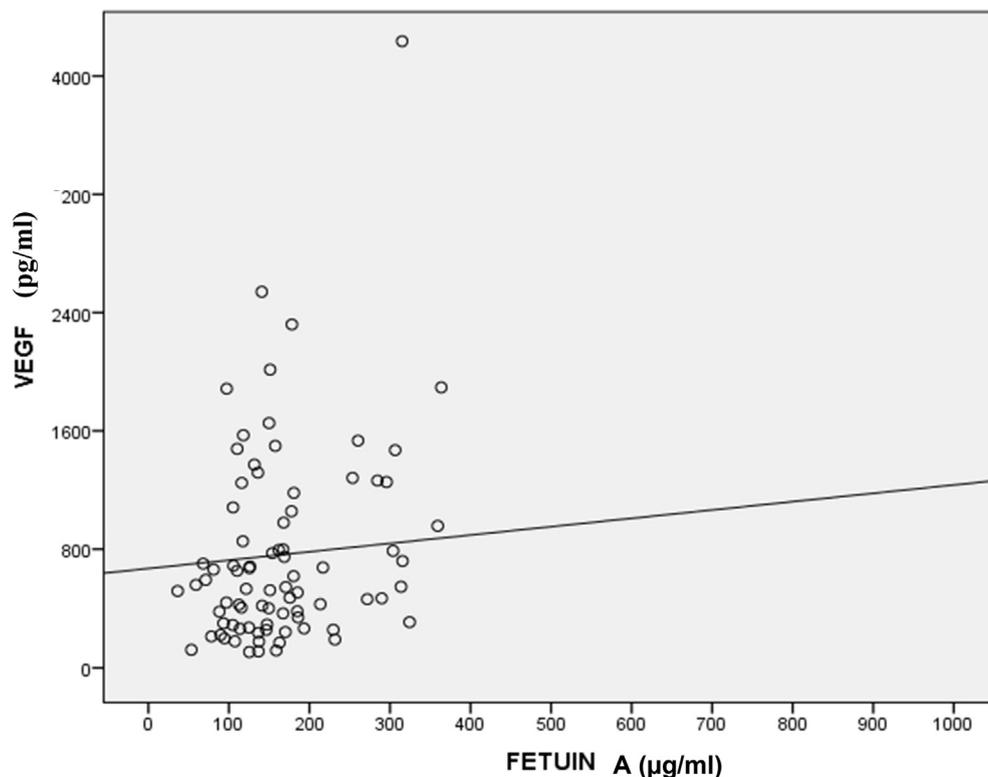


Fig. 1. Correlation analysis of Fetuin-A with VEGF in whole study population.

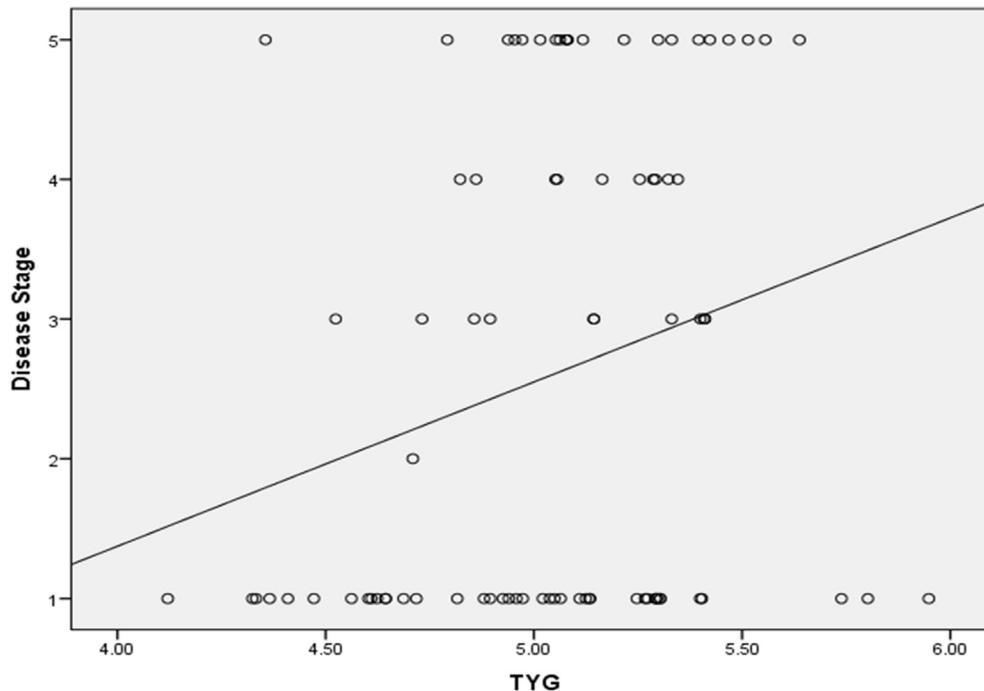


Fig. 2. Correlation analysis of TyG with disease severity in whole study population.

the adipose response to insulin signaling and glucose metabolism. It promotes efflux of glucose from the adipose tissue, where lipogenesis is inhibited by Fetuin-A. Earlier studies have shown that administration of Fetuin-A to rodents inhibited insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1 in rat liver and skeletal muscle, resulting in reduced insulin signaling and insulin resistance [23].

Fetuin-A can potentially be related to the lipid abnormalities that manifest in DR severity, and may an underrated role in developing insulin resistance in the earlier stages of the disease. Newly, cytological research exhibited that Fetuin-A effects vascular cell growth and producing angiogenic proteins by human perivascular fat cells [24].

We observed significantly elevated levels of VEGF in the cases. The VEGF levels also correlated positively to the Fetuin-A levels in the study. Previous evidences show that Fetuin-A can stimulate VEGF mRNA expression in both perivascular fat cells and retinal endothelial cells. In DR, it is well known that VEGF is elevated, and that it is a potent angiogenic factor. Correlation observed between Fetuin A and VEGF suggests how Fetuin-A can promote disease progression via stimulating VEGF. Thus, it could precipitate the proliferative state of DR, where active angiogenesis of unstable endothelium is a key feature [25]. It may be involved in the biological effects of increased lipolysis, insulin resistance, and angiogenic stimulation.

Hyperglycemia and abnormal lipid profiles in diabetes mellitus stimulate increased lipid peroxidation and ROS formation, an important mechanism in the pathogenesis of diabetic retinopathy. Our study observed a significant decline in the total antioxidant status of the cases. But, an increasing trend was observed in the MDA levels of the cases, though not statistically significant. There are not many studies on the relation of oxidative stress and Fetuin-A. We hypothesized that an increase in Fetuin-A could support the LDL-C accumulation in the retina, which might sustain the oxidative stress state. But, we could not determine any significant correlation between the oxidative stress markers and Fetuin-A. But, there is a possibility of Fetuin-A in promoting lipid-induced

oxidative stress in the diabetic retina. Because, it is well known that lipid peroxidation and lipid-induced inflammation are related to oxidative stress [26].

In the retina, accumulation of LDL-cholesterol occurs in the outer layer of the blood-retinal barrier, which is likely to get auto-oxidized [27]. The oxidized LDL also confers an important part in the oxidative stress pathway of DR pathogenesis. It can stimulate retinal cell apoptosis, causing retinal cell injury. This however could not be observed in our study. The role of Fetuin-A in lipid-related oxidative stress needs to be further studied in the future.

Our study has concluded that:[1] Type 2 Diabetes patients exhibited statistically significant increase in the serum levels of Fetuin-A, and VEGF, compared to type 2 diabetic patients without DR. Thus, the possibility of increased insulin resistance and angiogenesis participates in the development of diabetic retinopathy. Also, increased LDL-C, TC and decreased HDL-C, TAS in diabetic retinopathy has been observed.[2]Total antioxidant levels in the type 2 diabetic patients [NDR], was statistically greater, than in the patients with diabetic retinopathy. This suggests a significant stress due to free radicals, being predominant in the retinopathy state.[3] TyG index, the insulin resistance index, is associated positively with the severity of the disease.

These findings indicate that increase in Fetuin-A, aids in derangement of lipid metabolism, though exact mechanism is yet unclear. Conclusive evidence on Fetuin-A's function in propagating retinopathy, need to be studied in detail, with larger sample size. Literature so far, has pointed at its role in DR, via insulin resistance and lipid abnormalities. Targeting such molecules in DR, can hinder their functions, that alter metabolism, resulting in pathological consequences.

Disclosure

The authors declared no conflicts of interest. This study was funded by the JIPMER Intramural Research Grant (JIP/Res/Intramural/phs2/2017-18 dated 01/12/2017).

Acknowledgments

We would like to thank JIPMER, Puducherry, India for funding this study.

References

- [1] Frank R. Diabetic retinopathy and systemic factors. *Middle East Afr J Ophthalmol* 2015;22(2):151–7.
- [2] Corcóstegui B, Durán S, González-Albarrán MO, Hernández C, Ruiz-Moreno JM, Salvador J, et al. Update on diagnosis and treatment of diabetic retinopathy: a consensus guideline of the working group of ocular Health (Spanish society of diabetes and Spanish vitreous and retina society). *J Ophthalmol* 2017;2017:8234186.
- [3] Sayin N. Ocular complications of diabetes mellitus. *World J Diabetes* 2015;6(1):92–108.
- [4] Zhou ZW, Ju HX, Sun MZ, Fu QP, Chen HM, Ji HB, Ji F, Jiang DM. Serum fetuin-A levels are independently correlated with vascular endothelial growth factor and C-reactive protein concentrations in type 2 diabetic patients with diabetic retinopathy. *Clin Chim Acta* 2016;455:113–7.
- [5] Zhao Chunming, Hou Jiangping, Wang Shengxi, Jiang Rui. Relation of serum and vitreous concentrations of fetuin-A with diabetic retinopathy. *Med Sci Monit* 2015;21:1839–42.
- [6] Kim HW, Ko GJ, Kang YS, Lee MH, Song HK, Kim HK, Cha DR. Role of the VEGF 936 C/T polymorphism in diabetic microvascular complications in type 2 diabetic patients. *Nephrology* 2009;14(7):681–8.
- [7] Paine SK, Mondal LK, Borah PK, Bhattacharya CK, Mahanta J. Pro- and anti-angiogenic VEGF and its receptor status for the severity of diabetic retinopathy. *Mol Vis* 2017;23:356–63.
- [8] Saygili EI, Aksoy SN, Gurler B, Aksoy A, Erel O, Ozaslan M. Oxidant/antioxidant status of patients with diabetic and senile cataract. *Biotechnol Biotechnol Equip* 2010;24:1648–52.
- [9] Morales-Gonzalez JA, Morales-González A, Madrigal-Santillan EO. A master regulator of oxidative stress-the transcription factor Nrf2. *Intech*; 2016.
- [10] Arden GB, Sivaprasad S. Hypoxia and oxidative stress in the causation of Diabetic retinopathy. *Curr Diabetes Rev* 2011;7(5):291–304.
- [11] Zhao C, Hou J, Wang S, Jiang R. Relation of serum and vitreous concentrations of fetuin-A with diabetic retinopathy. *Med Sci Monit* 2015;21:1839–42.
- [12] Kang B, Yang Y, Lee EY, Yang HK, Kim HS, Lim SY, Lee JH, Lee SS, Suh BK, Yoon KH. Triglycerides/glucose index is a useful surrogate marker of insulin resistance among adolescents. *Int J Obes* 2017;41(5):789–92.
- [13] Sorrentino FS, Matteini S, Bonifazzi C, Sebastiani A, Parmeggiani F. Diabetic retinopathy and endothelin system: microangiopathy versus endothelial dysfunction. *Eye* 2018;32(7):1157–63.
- [14] WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363(9403):157–63.
- [15] Cetin EN, Bulgu Y, Ozdemir S, Topsakal S, Akin F, Aybek H, Yildirim C. Association of serum lipid levels with diabetic retinopathy. *Int J Ophthalmol* 2013;6(3):346–9.
- [16] Chang YC, Wu WC. Dyslipidemia and diabetic retinopathy. *Rev Diabet Stud* 2013;10(2–3):121–32.
- [17] Tohidi M, Baghbani-Oskouei A, Ahanchi NS, Azizi F, Hadaegh F. Fasting plasma glucose is a stronger predictor of diabetes than triglyceride-glucose index, triglycerides/high-density lipoprotein cholesterol, and homeostasis model assessment of insulin resistance: tehran Lipid and Glucose Study. *Acta Diabetol* 2018;55(10):1067–74.
- [18] Dabrowska AM, Tarach JS, Wojtysiak-Duma B, Duma D. Fetuin-A (AHSG) and its usefulness in clinical practice. Review of the literature. *Biomed Pap Med Fac Univ Palacky Olomouc Czechoslov* 2015;159(3):352–9.
- [19] Khalil H, Faizhalkuobaili. Elevated Fetuin - a level associated with atherogenic lipid profile in type 2 diabetes. *Int J Pharm Sci Rev Res* 2013;21(1):266–9.
- [20] Stefan N, Häring H-U. Circulating fetuin-A and free fatty acids interact to predict insulin resistance in humans. *Nat Med* 2013;19(4):394–5.
- [21] Strieder-Barboza C, Contreras GA. Fetuin-A modulates lipid mobilization in bovine adipose tissue by enhancing lipogenic activity of adipocytes. *J Dairy Sci* 2019;102(5):4628–38.
- [22] Vasques AC, Novaes FS, De Oliveira Mda S, Souza JR, Yamanaka A, Pareja JC, Tambascia MA, Saad MJ, Geloneze B. TyG index performs better than HOMA in a Brazilian population: a hyperglycemic clamp validated study. *Diabetes Res Clin Pract* 2011;93(3):e98–100.
- [23] Jensen MK, Jensen RA, Mukamal KJ, Guo X, Yao J, Sun Q, et al. Detection of genetic loci associated with plasma fetuin-A: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *Hum Mol Genet* 2017;26(11):2156–63.
- [24] D11 Siegel-Axel, Ullrich S, Stefan N, Rittig K, Gerst F, Klingler C, Schmidt U, Schreiner B, Randrianarisoa E, Schaller HE, Stock UA, Weigert C, Königsrainer A, Häring HU. Fetuin-A influences vascular cell growth and production of proinflammatory and angiogenic proteins by human perivascular fat cells. *Diabetologia* 2014;57(5):1057–66.
- [25] Zhou Z, Ju H, Sun M, Chen H, Ji H, Jiang D, Ji Y, Ji J. Serum fetuin-A concentrations are positively associated with serum VEGF levels in patients with newly diagnosed type 2 diabetes. *Endocr J* 2015;62(10):879–85.
- [26] Busik JV, Esselman WJ, Reid GE. Examining the role of lipid mediators in diabetic retinopathy. *Clin Lipidol* 2012;7(6):661–75.
- [27] Wu M, Chen Y, Wilson K, Chirindel A, Ihnat MA, Yu Y, Boulton ME, Szweda LI, Ma JX, Lyons TJ. Intraretinal leakage and oxidation of LDL in diabetic retinopathy. *Investig Ophthalmol Vis Sci* 2008;49(6):2679–85.