Research Article

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The role of zonulin in the pathogenesis of diabetic retinopathy

Diyabetik retinopatinin patogenezinde zonülinin rolü

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Abstract

Objectives: To evaluate serum levels of zonulin and interleukin 6 (IL-6) in type 2 diabetes mellitus (T2DM) patients complicated by diabetic retinopathy and to investigate the effect of intestinal permeability on diabetic retinopathy (DR) progression.

Materials and Methods: Eighty-nine patients with T2DM and 30 healthy controls aged between 18 and 80 years were enrolled. T2DM patients were grouped as without DR (n=30), with non-proliferative DR (n=29), and with proliferative DR (n=30). Serum zonulin and IL-6 levels were measured with the ELISA method.

Results: Zonulin levels showed no statistical difference by pairwise comparisons of groups, but IL-6 levels were significantly increased in non-proliferative and proliferative DR groups compared to the HC and without DR groups. Zonulin level was correlated with body mass index and IL-6 level; IL-6 level was correlated with duration of diabetes, fasting blood glucose and HbA1c. While duration of

diabetes and serum IL-6 levels had an independent effect on DR development, zonulin had no effect.

Conclusions: Serum zonulin levels cannot be used as a biomarker for the progression of the diabetic retinopathy complication.

Keywords: diabetes mellitus; diabetic retinopathy; inflammation; intestinal permeability; zonulin.

Öz

Amaç: Diyabetik retinopati komplikasyonuna sahip tip 2 diyabetes mellitus (T2DM) hastalarında serum zonulin ve interleukin 6 (IL-6) düzeylerini değerlendirmek ve bağırsak geçirgenliğinin diyabetik retinopati (DR) ilerlemesi üzerindeki etkisini araştırmaktır.

Gereç ve Yöntem: 18 ile 80 yaş aralığında T2DM hastalığına sahip 89 kişi ve 30 sağlıklı kontrol çalışmaya dahil edildi. T2DM hastaları DR bulunmayan (n=30), proliferatif olmayan DR (n=29) ve proliferatif DR (n=30) olarak gruplandırıldı. Serum zonulin ve IL-6 seviyeleri ELISA yöntemi ile ölçüldü.

Bulgular: Zonulin düzeyleri, grupların ikili karşılaştırmalarında istatistiksel bir fark göstermezken; IL-6 düzeyleri, sağlıklı kontrol ve DR bulunmayan gruplara kıyasla proliferatif olmayan ve proliferatif DR gruplarında önemli ölçüde arttı. Zonulin düzeyi, vücut kitle indeksi ve IL-6 seviyesi ile korelasyon mevcut iken IL-6 düzeyi diyabet süresi, açlık kan şekeri ve HbA1c ile korelasyon mevcuttu. Diyabet süresi ve serum IL-6 düzeylerinin DR gelişimi üzerinde bağımsız bir etkiye sahip olduğu, zonulinin etkisi olmadığı gözlenmiştir.

Sonuç: T2DM’da diyabetik retinopati komplikasyonunun ilerlemesi için serum zonulin düzeylerinin bir biyobelirteç olarak kullanılamayacağı sonucuna varıldı.

Anahtar Kelimeler: diyabetik retinopati; diyabetes mellitus; bağırsak geçirgenliği; zonulin; inflamasyon.

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Introduction

Diabetic retinopathy (DR) is one of the severe microvascular complications of diabetes mellitus (DM) and is the most prevalent cause of preventable visual loss in adulthood. Mainly chronic exposure to high blood glucose levels and other factors initiates activation of some biochemical pathways that finally give rise to retinal microvascular damage and dysfunction. These pathways include sorbitol accumulation, advanced glycation end-product formation, increased generation of reactive oxygen species, renin-angiotensin system upregulation, increased expression of vascular endothelial growth factor and inflammation, etc. [1].

Low-grade chronic inflammation is shown to have a prominent role in DR pathogenesis. There is an increase in the adherence of leukocytes from the initial stages of DR. Chemokines that regulate the activation and attraction of leukocytes such as monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1- α (MIP-1 α) and MIP-1 β were in DM patients. Also, inflammatory cytokines, such as interleukin-6 (IL-6), IL-8, IL-1 β , and tumor necrotizing factor- α (TNF- α) are increased in patients with DM and DR [2–5]. In addition, the roles of adipokines such as omentin-1 [6] and neuregulin-4 [7] in the development of DM and microvascular complications [8] were reported in several studies.

Recently increased attention has been paid to the relationship between chronic inflammation and gut microbiota. Altered gut microbiota composition or dysbiosis indicates an imbalance between commensal symbionts and pathobionts, significant elevation of pathobionts and decrease of beneficial butyrate-producing bacteria [9]. Many studies examined the link between gut microbiota and DM and revealed evidence of gut microbiota dysbiosis [10]. On the other hand, several human and animal studies implicated gut microbiota dysbiosis in pathogenesis of diabetic retinopathy [9] and also showed improvements of gut barrier function resulted in decreased retinopathy by the way of the gut-eye axis [11].

For monitoring intestinal permeability, a biomarker called zonulin, a 47 kDa protein that was revealed as prehaaptoglobulin-2, was shown to regulate tight junctions of enterocytes and in this way modulates intestinal permeability physiologically [12]. It was shown that the promoter region of the haptoglobulin gene that encodes zonulin is modulated by IL-6 via STAT3 activation and miR-18a induction [13]. Upregulation of zonulin leads to disassembly of tight junctions, uncontrolled antigen trafficking from the intestinal lumen to submucosa and consequently altered intestinal permeability, which gives rise to chronic inflammatory diseases [14, 15]. Nowadays, various studies indicated that circulatory levels of zonulin are elevated in several metabolic diseases such as

obesity, insulin resistance, metabolic syndrome, polycystic ovary syndrome, type 1 DM and T2DM [16–21].

Although IL-6 is shown to be one of the main regulators behind the pathological process in inflammatory ophthalmic diseases such as diabetic retinopathy [22], there is no report about IL-6 levels during the progression of DR simultaneously with zonulin. To date, knowledge about the interrelationship between intestinal permeability and clinical variables for patients with a relatively long duration of T2DM is sparse. In this respect, we aimed to evaluate serum zonulin levels along with systemic levels of IL-6 in T2DM patients with retinopathy complications and to investigate the correlation with metabolic and biochemical parameters.

Materials and methods

Study design

This study was designed as cross-sectional study and was approved by the Ethics Committee of the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey (16.04.2019-136). All participants were informed and written informed consent was obtained before the procedures according to the Helsinki Declaration.

A total of 119 subjects, between the ages of 18–80 years, were recruited to this study from the Endocrine and Metabolism Outpatient Clinic of Suleyman Demirel University Research and Practice Hospital, involving T2DM patients without DR (NDR; n=30), non-proliferative DR (NPDR; n=29), proliferative DR (PDR; n=30) and a healthy control group of 30 subjects (HC; n=30) without T2DM. Exclusion criteria included the presence of T1DM, acute or chronic infectious disease, renal or hepatic disease, rheumatological disease, cancer and regular usage of anti-inflammatory drugs. All participants' body mass indexes (BMI) were calculated using the measured body weight and height and; expressed in kilograms per square meter (kg/m^2); age, sex, time since diagnosis of DM (duration of DM), DM treatments received, existence of micro- and macrovascular complications of T2DM were recorded.

Ophthalmological examination

To determine the percentage and stage of diabetic retinopathy, the ophthalmic examinations of diabetic patients were performed by the same ophthalmologist in the Ophthalmology Outpatient Clinic. For pupil dilation, 1 or 2 drops of 0.5% tropicamide solution were applied into the eyes 30 min prior to examination. After appropriate dilatation, fundus examinations were performed by using Volk 90 dioptic (90D) ophthalmoscopic lens (Volk Optical Inc, USA). Patients were grouped according to the presence of DR as NDR and DR and severity as NPDR and PDR. Patients with DR were evaluated according to the Diabetic Retinopathy Severity Scale. If the lesions detected on fundus examination were limited to the retina, they were considered non-proliferative DR (NPDR), and if the retinal lesions progressed towards the vitreous, they were considered proliferative DR (PDR) [23].

Biochemical measurements

All biochemical analyses were performed at the Medical Biochemistry Laboratory of Suleyman Demirel University Research and Practice Hospital. After a minimum of 8 h of fasting, venous blood samples were collected into two blood collection tubes, an ethylene diamine tetra-acetic acid (EDTA) containing (BD Vacutainer K₂EDTA, 2 mL, 13 × 75 mm, NJ, USA) and a clot activator-serum separator tube (BD Vacutainer SST, 5 mL, 13 × 100 mm, NJ, USA). Glycosylated hemoglobin (HbA1c) determination was carried out by a high-performance liquid chromatography (HPLC) instrument (Bio-Rad, CA, USA). The serum specimen was separated after centrifugation at 3,000 rpm for 10 min and serum glucose, creatinine, alanine aminotransaminase (ALT), uric acid, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were measured on the Beckman Coulter AU 5800 chemistry analyzer (Beckman Coulter, Brea, USA). For low-density lipoprotein cholesterol (LDL-C) calculation, the Friedewald formula was used; $LDL-C = TC - (HDL-C + (TG/5))$. Serum zonulin and IL-6 levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (E-EL-H5560, E-EL-H0102; Elabscience, Wuhan, China). Detection limits of the ELISA kits were 0.47 ng/mL and 4.69 pg/mL, respectively.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15 (SPSS Inc, Chicago, IL, USA). The data were assessed for homogeneity of variance using the Levene test. Comparisons of data in groups which were not homogeneous were made using Kruskal-Wallis followed by Mann-Whitney U test and data which were distributed homogeneously were analyzed with one-way ANOVA. Data are reported as mean ± SEM. Categorical variables were compared using the chi-square test. Analysis of the correlations between parameters was performed using Pearson or Spearman's correlation analysis. Univariate and multivariate logistic regression

analyses were also performed. A p-value of <0.05 was considered statistically significant.

Results

Eighty-nine T2DM patients (30 patients with NDR, 29 patients with NPDR, 30 patients with PDR) and 30 HC were included in this study. Characteristics of the study population are summarized in Table 1. There were no significant differences between groups for sex distribution, BMI, ALT, uric acid, TG, HDL-C and TC levels ($p > 0.05$). Significant differences between groups were present for age ($p = 0.001$), duration of diabetes ($p < 0.001$), fasting blood glucose ($p < 0.001$), creatinine ($p < 0.001$), LDL-C ($p = 0.009$), HbA1c ($p < 0.001$), zonulin ($p = 0.049$) and IL-6 levels ($p < 0.001$).

Zonulin and IL-6 levels were significantly different between groups with assessment of the Kruskal Wallis test followed by Mann Whitney U test. Pairwise comparisons of groups for zonulin levels showed no statistical difference, but IL-6 levels were significantly increased in NPDR and PDR groups compared to the HC group. Additionally, IL-6 levels were significantly increased in NPDR and PDR groups compared to the NDR group (Table 1, Figures 1 and 2).

Significant positive correlations were found between zonulin with IL-6 (Figure 3) and BMI ($r = 0.201$, $p = 0.028$; $r = 0.336$, $p < 0.001$ respectively). Serum IL-6 level was also positively correlated with HbA1c, fasting blood glucose and duration of diabetes ($r = 0.299$, $p = 0.001$; $r = 0.364$, $p = 0.000$; $r = 0.422$, $p = 0.000$, respectively). HbA1c was positively correlated with fasting blood glucose and duration of

Table 1: Demographic, clinical and biochemical characteristics of study population (n=119).

	HC	NDR	NPDR	PDR	p-Value
Sex, male/female	13/17	12/18	15/14	13/17	0.827
Age, years	55.47 ± 1.33	53.27 ± 1.45	57.83 ± 1.40	61.27 ± 1.57*,**	0.001
Duration of diabetes, years	0.0	8.30 ± 1.19*	16.45 ± 1.16*,**	16.57 ± 1.27*,**	<0.001
BMI, kg/m ²	27.88 ± 0.65	30.61 ± 0.85	31.04 ± 1.16	30.27 ± 1.13	0.191
FBG, mg/dL	95.4 ± 1.33	183.3 ± 13.99*	213.0 ± 20.27*	228.36 ± 20.15*	<0.001
ALT, mg/dL	19.14 ± 1.33	25.33 ± 2.39	21.06 ± 1.73	18.56 ± 1.16	0.120
Uric acid, mg/dL	5.02 ± 0.24	5.25 ± 0.28	5.22 ± 0.24	5.88 ± 0.27	0.118
Creatinine, mg/dL	0.83 ± 0.02	0.88 ± 0.02	0.91 ± 0.02*	1.14 ± 0.04*,**	<0.001
TG, mg/dL	136.40 ± 13.61	160.37 ± 11.76	175.86 ± 18.27	176.50 ± 19.72	0.259
LDL-C, mg/dL	133.47 ± 5.32	124.47 ± 4.59	108.55 ± 4.80*	116.80 ± 6.15	0.009
HDL-C, mg/dL	50.70 ± 2.28	48.30 ± 2.20	48.17 ± 2.52	45.60 ± 2.01	0.467
TC, mg/dL	211.45 ± 7.11	204.70 ± 5.78	189.90 ± 8.95	196.40 ± 8.35	0.217
HbA1c, %	5.42 ± 0.07	8.39 ± 0.40*	8.68 ± 0.33*	8.96 ± 0.38*	<0.001
Zonulin, ng/mL	119.06 ± 10.89	100.63 ± 11.64	98.42 ± 18.17	138.82 ± 14.24	0.049
IL-6, pg/mL	5.41 ± 1.16	7.45 ± 1.49	16.25 ± 2.39*,**	16.71 ± 3.41*,**	<0.001

HC, healthy control; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; FBG, fasting blood glucose. * denotes the significant difference when compared to HC. ** denotes the significant difference when compared to NDR. Data are given as mean ± SEM.

Figure 1: Serum zonulin levels in study groups (HC, healthy control; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy).

Figure 2: Serum IL-6 levels in study groups (HC, healthy control; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy).

diabetes ($r=0.756$, $p=0.000$; $r=0.604$, $p=0.000$ respectively). Additionally, fasting glucose was positively correlated with duration of diabetes ($r=0.625$, $p=0.000$).

Independent effects of BMI, HbA1c, duration of diabetes, zonulin and IL-6 on DR development were investigated by multivariate logistic regression and it was found that duration of diabetes and serum levels of IL-6 had an independent effect on DR development, but zonulin did not have an effect (Table 2).

Discussion

In the present study, we evaluated the levels of zonulin as a gut permeability marker and IL-6 as an inflammatory marker in non-complicated and complicated DR associated with T2DM. The initial assessments of zonulin levels were significantly different between groups, while the pairwise comparisons of groups showed no significance; however, the lowest level was found in the NPDR group and the highest level was found in the PDR group. Serum IL-6 levels

were significantly increased in NPDR and PDR groups when compared to both HC and DM groups. These data show that the IL-6 levels were simultaneously increased with the progression of DM which may also represent the role of chronic inflammation in the diabetic retinopathy pathogenesis. To the best of our knowledge, this is the first study that assesses the serum zonulin and IL-6 levels in cases with T2DM and their possible association with the presence and severity of retinopathy.

In the literature, there are very few human studies that evaluate serum zonulin levels in T2DM patients [19, 20]. However, these studies did not include complicated diabetes with long duration, including newly diagnosed T2DM patients. Zhang et al. studied serum zonulin levels in subjects who were healthy, had impaired glucose tolerance and with newly diagnosed DM. The authors showed a progressive increase in serum zonulin level with the impairment of glucose tolerance and significantly highest levels in DM patients [19]. Jayashree et al. studied serum levels of lipopolysaccharide, IL-6, TNF- α and zonulin in healthy and newly diagnosed subjects. The authors found

Figure 3: Spearman correlation analysis between serum zonulin and IL-6 levels ($r=0.201$, $p=0.028$).

Table 2: Logistic regression analysis on diabetic retinopathy development.

	Odds ratio	p-Value	%95 CI
BMI	0.988	0.843	0.881–1.109
HbA1c	1.070	0.582	0.841–1.361
Duration of diabetes	1.189	<0.001	1.087–1.301
Zonulin	1.001	0.822	0.993–1.009
IL-6	1.068	0.041	1.003–1.118

CI, confidence interval.

that the levels of all parameters were significantly increased in the T2DM group [20]. While significantly increased IL-6 levels are consistent with Jayashere et al. [20], zonulin levels are contradictory. There were no significant differences in the T2DM group compared to healthy controls; furthermore, the zonulin levels were decreased in the NPDR group but finally increased in the PDR group. The main reason for this contradiction is the totally different construction of our study groups as healthy controls, DM, DM with non-proliferative retinopathy and DM with proliferative retinopathy. Also, in our study, diabetic patients were diagnosed in the past and monitored by a clinician for a period of time (8–16 years duration).

Human gut microbiota varies between individuals [24]. In addition, antidiabetic and antibiotic therapies and diet alterations were shown to reshape the gut microbiota. Metformin (dimethyl diguanide), a very common treatment for T2DM, is shown to affect intestinal microbiota positively and causes a decrease in zonulin levels when used in T2DM patients [25–27]. Nowadays, metformin usage is suggested with the purpose of preventing and treating aging-dependent leaky gut and inflammation by regulating intestinal microbiome, goblet cell count and also mucin biology [27]. In the present study, there was no significant

difference in metformin use between diabetic groups. High-fat diets are also related with decreased intestinal bacterial diversity and leaky gut [28]. The difficulty in interpreting these results is due to failure to evaluate gut microbiota in the present study and the fact that the participants did not consume a standard diet.

In the current study, the duration of DM, the level of fasting glucose, HbA1c and creatinine levels were steadily increased, and significantly higher levels were found in the PDR group compared to the HC and NDR groups (Table 1). This data is consistent with DR increasing proportionally to the disease duration in T2DM. Also, long-term exposure to high glucose levels increases the risk of vascular damage to the retina [1, 2].

Some limitations are present in this study. The cross-sectional nature of the study is the main one; serum zonulin and IL-6 levels of all participants were measured once. Other limiting factors are that a standard diet could not be applied to the participants included in the study and that gut microbiota was not evaluated.

Conclusion

IL-6 as a marker of chronic inflammation seems to be involved during the clinical progression of DR. The role of changes in serum zonulin level in the pathogenesis of DR could not be determined. However, further studies using a standardized diet and also evaluating gut microbiota may contribute to identifying the role of zonulin in DR pathogenesis.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.

References

- Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010;376:124–36.
- Wang W, Lo ACY. Diabetic retinopathy: pathophysiology and treatments. *Int J Mol Sci* 2018;19:1816.
- Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1 β , IL-6, TNF- α and VEGF have influence on the development of diabetic retinopathy. *Folia Med* 2011;53:44–50.
- Boss JD, Singh PK, Pandya HK, Tosi J, Kim C, Tewari A, et al. Assessment of neurotrophins and inflammatory mediators in vitreous of patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2017;58:5594–603.
- Ting DSW, Tan KA, Phua V, Tan GSW, Wong CW, Wong TY. Biomarkers of diabetic retinopathy. *Curr Diabetes Rep* 2016; 16:125.
- Tekce H, Tekce BK, Aktas G, Alcelik A, Sengul E. Serum omentin-1 levels in diabetic and nondiabetic patients with chronic kidney disease. *Exp Clin Endocrinol Diabetes* 2014;122:451–6.
- Kocak MZ, Aktas G, Erkus E, Yis OM, Duman TT, Atak BM, et al. Neuregulin-4 is associated with plasma glucose and increased risk of type 2 diabetes mellitus. *Swiss Med Wkly* 2019;149: w20139.
- Kocak MZ, Aktas G, Atak BM, Duman TT, Yis OM, Erkus E, et al. Is neuregulin-4 a predictive marker of microvascular complications in type 2 diabetes mellitus? *Eur J Clin Invest* 2020;50:e13206.
- Fernandes R, Viana SD, Nunes S, Reis F. Diabetic gut microbiota dysbiosis as an inflammaging and immunosenescence condition that fosters progression of retinopathy and nephropathy. *Biochim Biophys Acta Mol Basis Dis* 2019;1865:1876–97.
- De Kort S, Keszthelyi D, Masclee AAM. Leaky gut and diabetes mellitus: what is the link? *Obes Rev* 2011;12:449–58.
- Floyd JL, Grant MB. The gut–eye axis: lessons learned from murine models. *Ophthalmol Ther* 2020;9:499–513.
- Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet* 2000;355:1518–9.
- Brock M, Trenkmann M, Gay RE, Gay S, Speich R, Huber LC. MicroRNA-18a enhances the interleukin-6 mediated production of the acute-phase proteins fibrinogen and haptoglobin in human hepatocytes. *J Biol Chem* 2011;286:40142–50.
- Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 2011;91:151–75.
- Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers* 2016;4:e1251384.
- Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernandez-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. *PloS One* 2012;7:e37160.
- Zhang D, Zhang L, Yue F, Zheng Y, Russell R. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. *Eur J Endocrinol* 2015;172:29–36.
- Sapone A, De Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006;55:1443–9.
- Zhang D, Zhang L, Zheng Y, Yue F, Russell RD, Zeng Y. Circulating zonulin levels in newly diagnosed Chinese type 2 diabetes patients. *Diabetes Res Clin Pract* 2014;106:312–8.
- Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, Lakshmi BS, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem* 2014;388:203–10.
- Fasano A. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000 Res* 2020;9:69.
- Ulhaq ZS, Soraya GV, Wulandari LR. The role of IL-6-174 G/C polymorphism and intraocular IL-6 levels in the pathogenesis of ocular diseases: a systematic review and meta-analysis. *Sci Rep* 2020;10:1–20.
- 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.
- Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. *Science* 2005;308:1635–8.
- Palacios T, Vitetta L, Coulson S, Madigan CD, Lam YY, Manuel R, et al. Targeting the intestinal microbiota to prevent type 2 diabetes and enhance the effect of metformin on glycaemia: a randomised controlled pilot study. *Nutrients* 2020;12:2041.
- Adeshirlarijaney A, Gewirtz AT. Considering gut microbiota in treatment of type 2 diabetes mellitus. *Gut Microb* 2020;11: 253–64.
- Ahmadi S, Razazan A, Nagpal R, Jain S, Wang B, Mishra SP, et al. Metformin reduces aging-related leaky gut and improves cognitive function by beneficially modulating gut microbiome/goblet cell/mucin axis. *J Gerontol A Biol Sci Med Sci* 2020;75:9–21.
- Netto Candido TL, Bressan J, Alfenas RCG. Dysbiosis and metabolic endotoxemia induced by high-fat diet. *Nutr Hosp* 2018; 35:1432–40.