

Evaluation of sphingolipid metabolism on diabetic retinopathy

Ezgi Naz Ensari Delioğlu¹, Nagihan Uğurlu², Ebru Erdal³, Soheil Malekghasemi^{3,4}, Nurullah Çağıl²

Purpose: To evaluate the aqueous and serum levels of sphingolipid metabolism mediators such as sphingosine 1 phosphate (S1P), sphingosine kinase 1 (SK1), sphingosine kinase 2 (SK2), ceramide kinase (CK), and acid sphingomyelinase (ASM) which are thought to take part in diabetic retinopathy (DR) pathogenesis, and development and severity of diabetic retinopathy (DR) in patients with type 2 diabetes. **Methods:** A prospective cross-sectional study was conducted on type 2 diabetic and control patients who underwent cataract surgery. Three different subgroups, namely, non-diabetic retinopathy (NDR), non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR), were allocated and the S1P, SK1, SK2, CK, and ASM levels in the serum and aqueous humor samples of diabetic and control patients were evaluated. Kolmogorov-Smirnov test, Student's *t*-test, and Mann-Whitney U test were used for the statistical analysis of the study. **Results:** Among a total of 45 patients, including diabetic and control patients, the mean aqueous levels of SK1 ($P < 0.001$), SK2 ($P = 0.012$), ASM ($P = 0.006$), and CK ($P = 0.002$) were higher in all diabetic patients. The mean aqueous level of S1P was significantly higher in the PDR group than in other groups ($P = 0.003$). The mean aqueous levels of SK2 and ASM also increased in the NDR, NPDR, and PDR subgroups, respectively ($P < 0.001$). In addition, the mean serum levels of S1P, SK1, and ASM were higher in the diabetic patients ($P = 0.015$, $P = 0.034$, and $P = 0.006$, respectively). **Conclusion:** According to our findings, both aqueous and serum levels of S1P, SK1, and ASM and only the aqueous levels of SK2 and CK were higher in diabetic patients. This study suggested that sphingolipid metabolism may play an important role in DR pathogenesis.

Key words: Acid sphingomyelinase, ceramide kinase, diabetic retinopathy, sphingosine 1 phosphate, sphingosine kinase

Diabetic retinopathy (DR) is a microangiopathy affecting precapillary arterioles, capillaries, and venules in the retina and is one of the most important causes of blindness.^[1,2] The pathophysiology of DR is multifactorial and still not fully known. It is thought to develop due to endothelial cell dysfunction, damage to the blood-retina barrier, and hypoxia, while recent studies have shown that other factors such as inflammation, leukocytosis, and neurodegeneration can also play a role in development of DR.^[3] The increase in the prevalence of diabetes mellitus (DM) and the prolongation of the life expectancy of DM patients through new treatments have made DR one of the most important causes of preventable loss of vision. Therefore, many molecules are now being investigated for the pathogenesis and treatment of DR.^[4] Sphingolipids are considered as important regulators in the pathogenesis of cancers and many chronic diseases like diabetes due to their roles in pathophysiological processes such as the inflammatory signaling pathway, angiogenesis, proliferation, migration, apoptosis, and fibrosis.^[5,6]

Sphingolipids are formed via the metabolism of sphingomyelin, which is a component of the plasma membrane, or by *de novo* synthesis. Fragmentation of sphingomyelin is controlled by the sphingomyelinase (SM) enzymes. Sphingomyelinases are activated by many stimuli such as inflammation, cytokines, or growth factors; they hydrolyze sphingomyelin and create ceramide, which is a pro-inflammatory and pro-apoptotic secondary messenger.^[7] Acid sphingomyelinase (ASM) is the SM isoform, which makes retinal damage by increasing its level in diabetic hyperglycemia and dyslipidemia.^[8] In addition, inhibition of ASM has also been found to take part in preventing diabetes-related vascular damage.^[9]

Sphingosine 1 phosphate (S1P) is created by the phosphorylation via the sphingosine kinase (SK) enzymes of sphingosine, which is produced by the fragmentation of ceramide. Sphingosine 1 phosphate as pleiotropic lipid mediator has been an important research topic in neovascular ocular diseases since it suppresses sprouting angiogenesis and regulates vascular permeability. Dysfunction of the S1P

Access this article online

Website:

www.ijo.in

DOI:

10.4103/ijo.IJO_3724_20

Quick Response Code:



¹Basaksehir Cam and Sakura City Hospital, Department of Ophthalmology, İstanbul, ²Yıldırım Beyazıt University, Department of Ophthalmology, Ankara, ³Yıldırım Beyazıt University, Advanced Technologies Application and Research Center, Ankara, ⁴Hacettepe University, Department of Bioengineering, Ankara, Turkey

Correspondence to: Dr. Ezgi Naz Ensari Delioğlu, Başakşehir Çam ve Sakura Şehir Hastanesi, Başakşehir Olimpiyat Bulvarı Yolu, 34480 Başakşehir/İstanbul, Türkiye. E-mail: enazensari@gmail.com

Received: 19-Dec-2020

Revision: 07-Mar-2021

Accepted: 04-May-2021

Published: 29-Oct-2021

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

Cite this article as: Ensari Delioğlu EN, Uğurlu N, Erdal E, Malekghasemi S, Çağıl N. Evaluation of sphingolipid metabolism on diabetic retinopathy. Indian J Ophthalmol 2021;69:3376-80.

signaling system results in various vascular defects, such as inappropriate vascularization and vascular leakage in developing retina due to its pro-inflammatory, proangiogenic, and profibrotic properties.^[10,11]

Sphingosine kinase is the key enzyme in S1P production. It controls via phosphorylation the sphingolipid rheostat between ceramide, and sphingosine with pro-apoptotic effects, and S1P with an anti-apoptotic effect.^[12] It has two isoforms named SK 1 and 2. The tissue distribution of the SK isoforms is different; SK1 is more common in the lungs and spleen, while SK2 is more common in the heart, brain, and liver.^[13] The activation of SK 1 is associated with cell proliferation, survival, migration, differentiation, angiogenesis, and inflammation. It has been reported to play a role in growth, metastasis, and chemoresistance in various cancers.^[11] However, the function of SK 2 has been investigated less frequently.^[14]

Ceramide kinase (CK) phosphorylates ceramide and forms ceramide 1 phosphate (C1P). Although its functions in the retina are not fully known, C1P has been shown to induce the proinflammatory cascade, decrease apoptosis, stimulate angiogenesis, and increase cell survival. Furthermore, according to *in vitro* studies, high secretion level of C1P has been found in damaged cells.^[15]

The pathways in which given bioactive lipid mediators play a role are directly related to DR pathogenesis. Although descriptive biochemical studies have been conducted to investigate the relationship between DR and sphingolipid metabolism by *in vitro* and animal studies, few studies have been performed in humans in the literature. The aim of this study was to evaluate the aqueous and serum levels of S1P, SK1, SK2, ASM, and CK among the mediators and enzymes related to sphingolipid metabolism, which are thought to take part in the DR pathogenesis, and investigate their relationship with development and severity of DR in patients with type 2 DM.

Methods

The study was conducted in accordance with the Helsinki Declaration and approved by the University Clinical Studies Ethics Committee. All patients were informed on the study and an informed consent form was obtained. A total of 30 type 2 diabetic patients and 15 control group patients who underwent cataract surgery without complication at the University's Department of Ophthalmology during April and May 2018 were included in this study with a prospective cross-sectional design. Diabetic patients were divided into 3 subgroups named non-diabetic retinopathy (NDR), non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR) according to the ophthalmologic examination and fundus fluorescein angiography images on the date they were included in the study. Patients who had undergone any ocular surgery or experienced ocular trauma, had a history of intraocular anti-VEGF injection or treated with laser in the last 3 months, intravitreal steroid injection or topical/systemic steroid use, who had retinal pathology other than DR, corneal pathology, uveitis or glaucoma, history of cancer, chronic obstructive lung disease, coronary artery disease, myocardial infarction or cerebrovascular incident, and who were under forty years

old, suffering from rheumatic disease and neurodegenerative disease such as Alzheimer's, and also those did not accept the informed consent form were excluded from the study. Preoperative visual acuity and intraocular pressure were measured in all patients and anterior and posterior segment examinations were conducted by biomicroscope. In addition, hemoglobin A1C (HbA1c) levels of all patients measured in the last 3 months were recorded.

Five ml venous blood samples were taken from all patients before starting the surgery and centrifuged at 1500–2000 × g for 10 min after placement in 13 × 100 tubes containing a separator gel and then left to rest for 30 min. The obtained serum samples were put into Eppendorf tubes and stored in the freezer at –80°C.

All patients were operated on by the same experienced surgeon. After cleaning the surgical site and administration of topical anesthesia, the anterior chamber was entered through the limbus, and 0.05–0.1 ml aqueous humor was taken with a 27-gauge cannula. The aqueous humor samples were transferred to Eppendorf tubes and stored in the freezer at –80°C.

S1P, SK1, SK2, CK, and ASM levels were evaluated in the aqueous humor and serum samples taken from diabetic and control patients. All samples were simultaneously thawed and brought to room temperature. The aqueous humor samples were diluted with balanced salt solution (BSS) at a ratio of 1:10 to ensure an amount sufficient for analysis. Serum samples were studied for S1P, SK1, SK2, and CK without dilution, but were diluted with phosphate buffer saline (PBS) at a ratio of 1:100 according to the kit instructions for ASM. SK1 and SK2 (SunRed, China), and CK and ASM (Cloud-Clone Corp., USA) were studied with the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method and S1P (Cloud-Clone Corp., USA) was studied with the competitive inhibition ELISA method using the relevant assay kits and a microplate reader device (Clariostar, BMG Labtech, Germany). All aqueous and serum samples were studied three times and the mean value and standard deviation (S.D.) was recorded. Results were determined by applying the absorbance values to the formulas obtained from the standard calibration curves of each kit.

The statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 20 software (IBM SPSS Inc., Chicago, IL). The normal distribution of the data was evaluated with the Kolmogorov-Smirnov test. Student's *t*-test (for numerical variables exhibiting a normal distribution) and the Mann-Whitney U test (for numerical variables not distributed normally) were used in the determination of the variables showing a difference between the control and diabetes groups. The ANOVA test (*post hoc*: Bonferroni-corrected Student's *t*-test) was used for numerical variables exhibiting a normal distribution and the Kruskal-Wallis test (*post hoc*: Bonferroni-corrected Mann-Whitney U test) for numerical variables not distributed normally in the determination of variables showing a difference between the DM subgroups and the control group. The Chi-Square and Fisher's Exact Chi-Square tests were used in the comparison of the categorical data. A *P* value < 0.05 was accepted as significant in the statistical analyses.

Results

A total of 45 patients consisting of 30 patients with type 2 DM and 15 healthy patients who had undergone cataract surgery without any complication were included in the study. The mean age of the 45 patients was 65.4 ± 9.4 years, and 23 patients (51.1%) were female. No significant difference was found between the diabetes patients and the control group in terms of mean age and gender distribution. The mean HbA1c level was significantly higher in patients with diabetes than the control group ($P < 0.001$) [Table 1].

In the diabetic patient group, 11 patients (36.7%) were assigned to each of the NDR and NPDR subgroups and 8 patients (26.7%) were allocated to PDR subgroup. The mean age and gender distribution in the diabetes subgroups were not significantly different than the control

group ($P = 0.857$). HbA1c levels were found to be higher in the NDR subgroup than the control group and also higher in the NPDR and PDR subgroups than the NDR subgroup ($P < 0.001$) [Table 2].

The distribution of the aqueous levels of the sphingolipid metabolism parameters in the groups is presented in Table 3. The mean aqueous SK1, SK2, ASM, and CK levels were higher in all diabetic patients compared to the control group ($P < 0.001$, $P = 0.012$, $P = 0.006$, and $P = 0.002$, respectively). The mean aqueous level of S1P was similar to the control group in all patients with diabetes ($P = 0.512$); however, it was significantly higher in the PDR subgroup than the NDR, NPDR, and control group ($P = 0.003$). The mean aqueous levels of SK2, ASM, and CK were increasingly higher in the PDR, NPDR, and NDR subgroups, respectively, in parallel with the severity of DR ($P < 0.001$, $P < 0.001$, and $P = 0.010$). In addition, the mean aqueous levels of SK1, SK2, and CK were significantly higher in all diabetes subgroups than the control group ($P < 0.001$, $P < 0.001$, and $P = 0.010$, respectively).

The distribution of serum levels of the sphingolipid metabolism parameters in the groups is presented in Table 4. The mean serum levels of S1P, SK1, SK2, and ASM in the diabetic patients were higher than in the control group ($P = 0.015$, $P = 0.034$, $P = 0.379$, and $P = 0.006$, respectively). The mean serum levels of S1P, SK2, and CK showed no significant difference among the diabetes subgroups. Finally, the mean serum level of SK1 was significantly higher in the PDR and NPDR subgroups

Table 1: Demographic and clinical features of diabetes and control groups

	Control group <i>n</i> =15, (%)	Diabetes group <i>n</i> =30, (%)	<i>P</i>
Age	63.73±12.07	66.2±7.77	0.411
Gender			
Female	6 (40.0)	17 (56.7)	
Male	9 (60.0)	13 (43.3)	
HbA1c	5.63±0.28	7.86±1.60	<0.001

Table 2: Demographic and clinical features of control and diabetes subgroups

	Control group <i>n</i> =15, (%)	NDR group <i>n</i> =11, (%)	NPDR group <i>n</i> =11, (%)	PDR group <i>n</i> =8, (%)	<i>P</i>
Age	63.73±12.07	66.00±6.18	66.91±9.99	65.5±7.17	0.857
Gender					
Female	6 (40.0)	8 (72.7)	3 (27.3)	6 (75.0)	0.074
Male	9 (60.0)	3 (27.3)	8 (72.7)	2 (25.0)	
HbA1c		6.98±0.86	8.12±1.44	8.70±2.10	<0.001

Table 3: Distribution of the aqueous levels of the sphingolipid metabolism parameters in the groups

Aqueous levels	Control group <i>n</i> =15	Diabetes group <i>n</i> =30	NDR group <i>n</i> =11	NPDR group <i>n</i> =11	PDR group <i>n</i> =8	<i>P</i> (Control vs diabetes group)	<i>P</i> (Control vs diabetes subgroups)
S1P (ng/ml)	28.59±0.85	29.39±1.82	28.59±0.68	28.75±1.77	31.37±1.60	0.512	0.003
SK1 (ng/ml)	24.43±1.92	31.69±2.17	30.19±1.76	32.46±1.61	32.48±2.51	<0.001	<0.001
SK2 (ng/ml)	9.49±0.55	11.99±1.71	10.55±0.88	11.94±1.02	13.86±1.52	0.012	<0.001
ASM (ng/ml)	0.59±0.18	0.91±0.44	0.66±0.32	0.942±0.31	1.23±0.55	0.006	<0.001
CK (ng/ml)	41.43±6.63	56.38±15.8	52.79±20.6	57.11±11.33	60.33±14.31	0.002	0.010

Table 4: Distribution of the serum levels of the sphingolipid metabolism parameters in the groups

Serum Levels	Control group <i>n</i> =15	Diabetes group <i>n</i> =30	NDR group <i>n</i> =11	NPDR group <i>n</i> =11	PDR group <i>n</i> =8	<i>P</i> (Control vs diabetes group)	<i>P</i> (Control vs diabetes subgroups)
S1P (ng/ml)	255.54±110.44	476.52±171.42	458.27±148.16	472.80±187.15	506.74±196.88	0.015	0.020
SK1 (ng/ml)	21.61±15.67	40.17±24.00	36.12±22.9	41.44±22.02	44.27±29.74	0.034	0.045
SK2 (ng/ml)	7.89±7.24	10.21±7.6	12.12±10.51	9.40±5.47	8.60±5.13	0.379	0.557
ASM (ng/ml)	15.85±3.18	19.73±3.43	19.75±2.47	22.02±2.51	16.86±3.56	0.006	<0.001
CK (ng/ml)	29.42±6.05	30.59±10.43	34.05±12.37	30.48±9.00	25.98±8.4	0.594	0.213

than the NDR subgroup and also significantly higher in the NDR subgroup than the control group ($P = 0.045$).

Discussion

The aqueous levels of S1P, SK1, SK2, CK, and ASM and the serum levels of SK1, SK2, and CK were measured for the first time in the literature through this study. The serum levels of patients with type 2 DM were also compared with the control group, and the role of sphingolipid metabolism in the pathogenesis of DR was investigated. While SK2 and CK levels were found to be high only in the aqueous humor in diabetic patients, the S1P, SK1, and ASM levels in the serum were also found to be higher in diabetic patients than in the control group.

The aqueous level of S1P was higher in the PDR subgroup than in the other diabetes subgroups and control group. The serum level of S1P was found to be higher in diabetics, but no significant difference was found among diabetes subgroups. Various studies have reported that the plasma level of S1P in patients with Type 2 diabetes is higher than in healthy individuals and is positively correlated with the HbA1c level.^[16,17] Besides, various studies have reported that the SK1/S1P pathway plays a crucial role in the pathogenesis of diabetic nephropathy and other microvascular complications of diabetes as well as it can lead to fibrosis by stimulating proliferation and matrix formation in mesangial cells.^[18-20] However, there is no previous study evaluating the level of S1P in the aqueous humor in the literature. The indifference in serum level of S1P between the PDR and NPDR subgroups can be explained by the fact that the two subgroups are similar in terms of HbA1c levels. The plasma level of S1P can also be affected by factors such as the total body fat mass and body mass index.^[17] While the higher serum level of S1P in the patients with diabetes than in the control group indicates DM-related systemic microvascular damage, a high aqueous humor level found only in the PDR group signifies its role in the advanced stages of DR and neovascularization. It is also possible that the aqueous level of S1P in the NPDR subgroup was not as high as expected due to the severity of retinopathy in these patients. Another reason why the aqueous S1P level was high only in the PDR subgroup may be related to the fact that the S1P lyase, induced by cellular stress, irreversibly degrades S1P. Abu El-Asrar *et al.*^[21] reported that sphingosine 1 phosphate receptor 1 (S1PR1) and S1P lyase enzyme levels in the vitreous samples of patients with PDR were higher than in the control group. However, the direction of S1P production-destruction balance shifted in the NPDR and NDR subgroups is not yet known.

In this study, aqueous and serum levels of the SK1 and aqueous level of the SK2 were found to be higher in patients with diabetes compared to the control group. The serum levels of SK2 were similar in diabetes and control groups, but the aqueous levels were found to be high in diabetes subgroups in parallel with the severity of the DR. Despite descriptive *in vivo* and *in vitro* biochemical studies explaining the possible mechanisms of the SK enzyme or the SK1 isoform in DR pathogenesis, there is no study investigating enzyme levels in humans. In addition, the relationship between SK2 and DR is not known. Maines *et al.*^[22] have reported that SK expression is increased in diabetic rats, and retinal vascular leakage is also decreased with SK enzyme inhibition. Wang *et al.*^[23] reported that hyperglycemia in a human umbilical endothelial cell culture

caused inflammation by increasing the SK and SK1 enzyme activity, not SK2. Although this appears to be in contrast to the high SK2 aqueous levels according to our findings, it may be explained by different effect sites of the SK isoforms.^[14] The results of our study show that SK1 is closely associated with the systemic damage of diabetes, and SK2 is more likely involved in DR pathogenesis instead of having a systemic effect.

The aqueous and serum ASM levels of the diabetic patients were higher than in the control group, and the aqueous levels of ASM were also higher in the diabetes subgroups in parallel with DR severity.

The main forms of the sphingomyelinase enzyme are ASM and neutral SM leading to ceramide production and cell death in response to cellular stress. Given these two enzymes have different pH values, the ASM can act better at lower pH value. Therefore, ASM can play an important role in the diabetic retina where an acidic environment is caused by hyperglycemia and hypoxia.^[9] Thus, we investigated the level of the ASM form of SM enzymes.

Various studies have reported that ASM and ceramide levels increase in diabetes. Opreanu *et al.*^[9] reported that the release of ASM increased in the retina of diabetic rats with acellular capillaries due to ischemia-reperfusion damage is less frequent in rats with suppressed ASM secretion, as well. Kady *et al.*^[24] found that ASM release increased in the plasma, circulating angiogenic cells of patients with diabetes, and postmortem retinal endothelial cells of diabetic patients. Wu *et al.*^[25] showed that increased ASM in the retina of rats with acute retinal ischemia caused retinal damage leading to increased ceramide and TNF- α , whereas suppression of ASM release decreased this damage. For the first time, the aqueous level of ASM was measured in this study and its high serum and aqueous levels in DM patients were found in parallel with the results of other studies.^[9,24,25] All of these results indicate the important role of ASM in the pathogenesis of DR.

Aqueous CK levels were higher in diabetic patients than control group and also in PDR and NPDR subgroups than NDR subgroup, whereas serum CK levels showed no difference among groups. Ouro *et al.*^[26] reported that C1P, the product of the CK enzyme, stimulates the VEGF secretion of macrophages. Besides, by activating phospholipase A2, C1P could stimulate the synthesis of arachidonic acid and prostaglandin stronger than ceramide.^[6] Miranda *et al.*^[27] reported that C1P regulates the proliferation of retinal photoreceptors and also stimulates the survival and differentiation of these cells. However, there is no study investigating the relationship between CK or C1P with DR in the literature. The similar serum CK levels of the groups and the high aqueous level of CK in the diabetic patients indicate increased local production and release of CK in diabetic retina.

Ceramide inhibits many intermediate products in the insulin signaling pathway. Although ceramide levels in insulin resistance patients are higher than in healthy controls, this difference is less than two-fold.^[28] Priyadarsini *et al.*^[29] have reported that the ceramide level increased and sphingomyelin level decreased in the corneas of type 1 and type 2 DM patients. Gorska *et al.*^[30] reported that levels of sphingosine and sphinganine produced from ceramide breakdown were higher in patients with type 2 DM than in healthy controls. This suggests that both the production

and breakdown pathways of ceramide metabolism are active in diabetic patients. In addition, it explains why both ASM and CK enzymes in this study are elevated in diabetic patients. Another enzyme inherent to ceramide metabolism is the glucosylceramide synthase which forms glucosylceramide. Fox *et al.*^[31] found that cellular ceramide levels were decreased with a corresponding increase in glucosylceramides; however, they indicated that glucosylceramide synthase was unaltered in the diabetic retina. As it is seen, in the literature, there are different results relevant to the mediators in sphingolipid metabolism; therefore the studies that evaluate lots of mediators at the same time will be very beneficial.

Conclusion

This study has shown that sphingolipid metabolism may play an important role in DR pathogenesis, however, further prospective studies with long follow-up periods, larger number of patients, and researching vitreous samples are required. Understanding the relationship between sphingolipid metabolism and DR development will be inspiring in terms of the prevention of disease progression and the development of effective treatment strategies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

- Aiello LP. Angiogenic pathways in diabetic retinopathy. *N Engl J Med* 2005;353:839-41.
- Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res* 2007;2007:95103.
- Bandello F, Zarbin MA, Lattanzio R, Zucchiatti I. Management of diabetic retinopathy. *Dev Ophthalmol* 2017;60:16-27.
- Cheung N, Mitchell P, Wong, T. Diabetic retinopathy. *Lancet* 2010;376:124-36.
- Airola M, Hannun Y. Sphingolipid metabolism and neutral sphingomyelinases. *Handb Exp Pharmacol* 2013;215:57-76.
- Hait NC, Maiti A. The role of sphingosine-1-phosphate and ceramide-1-phosphate in inflammation and cancer. *Mediators Inflamm* 2017;2017:1-17.
- Nixon GF. Sphingolipids in inflammation: Pathological implications and potential therapeutic targets. *Br J Pharmacol* 2009;158:982-93.
- Chakravarthy H, Navitskaya S, O'Reilly S, Gallimore J, Mize H, Beli E, *et al.* Role of acid sphingomyelinase in shifting the balance between proinflammatory and reparative bone marrow cells in diabetic retinopathy. *Stem Cells* 2016;34:972-83.
- Opreanu M, Tikhonenko M, Bozack S, Lydic TA, Reid GE, McSorley KM, *et al.* The unconventional role of acid sphingomyelinase in regulation of retinal microangiopathy in diabetic human and animal models. *Diabetes* 2011;60:2370-8.
- Caballero S, Swaney J, Moreno K, Afzal A, Kielczewski J, Stoller G, *et al.* Anti-sphingosine-1-phosphate monoclonal antibodies inhibit angiogenesis and sub-retinal fibrosis in a murine model of laser-induced choroidal neovascularization. *Exp Eye Res* 2009;88:367-77.
- Obinata, H, Hla T. Sphingosine 1-phosphate and inflammation. *Int Immunol* 2019;31:617-25.
- Haass NK, Nassif N, McGowan EM. Switching the sphingolipid rheostat in the treatment of diabetes and cancer comorbidity from a problem to an advantage. *Biomed Res Int* 2015;2015:165105.
- Ng ML, Wadham C, Sukocheva OA. The role of sphingolipid signalling in diabetes-associated pathologies [review]. *Int J Mol Med* 2017;39:243-52.
- Kwong EK, Li X, Hylemon PB, Zhou H. Sphingosine kinases/ sphingosine 1- phosphate signaling in hepatic lipid metabolism. *Curr Pharmacol Rep* 2017;3:176-83.
- Mesev EV, Miller DS, Cannon RE. Ceramide 1-phosphate increases p-glycoprotein transport activity at the blood-brain barrier via prostaglandin E2 signaling. *Mol Pharmacol* 2017;91:373-82.
- Randriamboavonjy V, Badenhoop K, Schmidt H, Geisslinger G, Fisslthaler B, Fleming I. The S1P2 receptor expressed in human platelets is linked to the RhoA-Rho kinase pathway and is down regulated in type 2 diabetes. *Basic Res Cardiol* 2009;104:333-40.
- Kowalski GM, Carey AL, Selathurai A, Kingwell BA, Bruce CR. Plasma sphingosine-1-phosphate is elevated in obesity. *PLoS One* 2013;8:e72449.
- Chen C, Gong W, Li C, Xiong F, Wang S, Huang J, *et al.* Sphingosine kinase 1 mediates AGEs-induced fibronectin upregulation in diabetic nephropathy. *Oncotarget* 2017;8:78660-76.
- Huang K, Huang J, Chen C, Hao J, Wang S, Huang J, *et al.* AP-1 regulates sphingosine kinase 1 expression in a positive feedback manner in glomerular mesangial cells exposed to high glucose. *Cell Signal* 2014;26:629-38.
- Lan T, Liu W, Xie X, Xu S, Huang K, Peng J, *et al.* Sphingosine kinase-1 pathway mediates high glucose- induced fibronectin expression in glomerular mesangial cells. *Mol Endocrinol* 2011;25:2094-105.
- Abu El-Asrar AMA, Nawaz MI, Mohammad G, Siddiquei MM, Alam K, Mousa A, *et al.* Expression of bioactive lysophospholipids and processing enzymes in the vitreous from patients with proliferative diabetic retinopathy. *Lipids Health Dis* 2014;13:1-8.
- Maines LW, French KJ, Wolpert EB. Pharmacologic manipulation of sphingosine kinase in retinal endothelial cells: Implications for angiogenic ocular diseases. *Invest Ophthalmol Vis Sci* 2006;47:5022-31.
- Wang L, Xing XP, Holmes A, Wadham C, Gamble JR, Vadas MA, *et al.* Activation of the sphingosine kinase-signaling pathway by high glucose mediates the proinflammatory phenotype of endothelial cells. *Circ Res* 2005;97:891-9.
- Kady N, Yan Y, Salazar T, Wang Q, Chakravarthy H, Huang C, *et al.* Increase in acid sphingomyelinase level in human retinal endothelial cells and CD34+circulating angiogenic cells isolated from diabetic individuals is associated with dysfunctional retinal vasculature and vascular repair process in diabetes. *J Clin Lipidol* 2017;11:694-703.
- Wu BX, Fan J, Boyer NP, Jenkins RW, Koutalos Y, Hannun YA, *et al.* Lack of acid sphingomyelinase induces age-related retinal degeneration. *PLoS One* 2015;10:e0133032.
- Ouro A, Arana L, Riazzy M, Zhang P, Gomez-Larrauri A, Steinbrecher U, *et al.* Vascular endothelial growth factor mediates ceramide 1-phosphate-stimulated macrophage proliferation. *Exp Cell Res* 2017;361:277-83.
- Miranda GE, Abrahán CE, Agnolazza DL, Politi Le, Rotstein NP. Ceramide-1-phosphate, a new mediator of development and survival in retina photoreceptors. *Invest Ophthalmol Vis Sci* 2011;52:6580-8.
- Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 2006;45:42-72.
- Priyadarsini S, Sarker-Nag A, Allegood J, Chalfant C, Karamichos D. Description of the sphingolipid content and subspecies in the diabetic cornea. *Curr Eye Res* 2015;40:1204-10.
- Gorska M, Dobrznyn A, Baranowski M. Concentrations of sphingosine and sphinganine in plasma of patients with type 2 diabetes. *Med Sci Monit* 2005;11:35-8.
- Fox TE, Han X, Kelly S, Merrill AH Jr, Martin RE, Anderson RE, *et al.* Diabetes alters sphingolipid metabolism in the retina: A potential mechanism of cell death in diabetic retinopathy. *Diabetes* 2006;55:3573-80.