Journal of Research Administration
Society of Research Administrators International

PLASMA METABOLOMICS DETECTION DETERMINES THE METABOLITE PROFILING ASSOCIATED WITH DIABETIC RETINOPATHY AND PROGRESSION OF DISEASE

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Abstract
Diabetic retinopathy (DR) is a major complication of diabetes, as a consequence it result into microvascular retinal changes on account of excess glycemic levels over a long time. Metabolomics profiling is one of the rapidly evolving detection strategy and is used to recognize the metabolites originated in serum and are responsible to investigate retinopathy progression in type-2 diabetic (T2D) patients. In this study, the serum metabolites concentrations was quantified in (T2D) patients.Diabetic patients were further categorized into three sub-groups based on the status of their complications: non-DR (NDR, n = 141), non-proliferative DR (NPDR, n = 124), and proliferative DR (PDR, n = 52) groups.The studied results revealed the concentrations of 62 metabolites of the NDR vs DR group, 53 metabolites of the NDR vs NPDR group, and 30 metabolites of the NDR vs PDR group were found to be significantly different. Of these metabolites, only sixteen metabolites were selected as common and specifically observed in NPDR and PDR groups. Among them, only three remaining metabolites comprising of total DMA, tryptophan, and kynurenine were act as potential maker system of retinopathy progression in T2D patients. Additionally, other metabolites such as carnitines, several amino acids, and phosphatidylcholinesalso showed distinguishing feature of potential marker system.
The metabolites recognized in this study will provide pathway to understand mechanisms concerning to retinopathy development and its progression in T2D patients, as it is helpful to diagnosis disease development at earlier stages and provide a valuable insight to develop appropriate therapeutic measures.
Keywords: metabolites, diabetic retinopathy, diabetes, metabolomics,

1. Introduction

Diabetic retinopathy (DR) is one of the most common microvascular complication of diabetes (Sumarriva et al. 2019), as a result it leads to retinal changes prominence in adults worldwide (Ogurtsova et al. 2017). The worldwide existence of diabetic cases is estimated to be increases as it is predictable through a series of population surveys at large-scale and itshows that diabetes prevalence has risen sharply to 9-12% in last few years especially in China where more than one million people are affected (Wang et al. 2017). The increment in number of diabetic cases in china can elevate its rank in the world and it would be estimated that more than 1.6million peoples were affected seriously with blindness on account of diabetic retinopathy. Therefore there must be a therapeutic dimensions and intervention for early detection of diabetic retinopathy with respect to significant decrement clinically. With respect to progression and development of diabetic

retinopathy,glycemic level and duration of disease are the two major systemic risk factors prominently involved in such events (Ting, 2016).However, these highlighted risk factors couldn't elucidateinconsistency that explore the disease retinopathy existence and their rate of progression in different diabetic patients, significantly deduce that other risk factors may also involve in this event (Lachin et al. 2008). Several studies decode that so many diabetic patients have more prone to develop diabetic retinopathy with intensive control of glycemic index as compared to those patients with poor chance to control blood glycemic index. This facts is evidently proven with the existence of different kinds of metabolites as metabolic memory in plasma which might be contribute differential kind of retinal cell behavior especially in controlled diabetic patients (Chen et al. 2016). Although the metabolic memory term indicate epigenetic modification in a continual fashion as it is triggered due to inadequate glycemic control surprisingly regulate in the early stage of diabetic patients.

Such diabetic individuals are still under surveillance to investigate the progression of diabetic complications even after their glycemic index hits the normal range for a period of time (Pirola et al. 2010). On account of gene to environment interaction might influences the differential expression of metabolites as it causes altered retinal cell behavior by Pirola et al. (2010). Identifying of such epigenetic factors in diabetic retinopathy patients could be preliminary indication for early diagnosis and treatment of disease progression (Nunes et al. 2013).Henceforth,the early detection of such biomarkers that causedeviations in the progression of diabetic retinopathy consequently become so much essential as these biomarkers will explore unknown pathogenic pathways, thus it may serve as novel approaches for the early diagnosis and intrusion of diabetic retinopathy (Tan et al. 2013).

Metabolomics, a combinatorial biological science concerning with metabolites as biomarkers associated with metabolic disease (Holmes et al. 2008). Diabetic retinopathy is a complex metabolic disease relates to the interaction between the environmental and genetic factors (Kuo et al. 2014), thus the identification of distinct metabolic signature concerning to diabetes retinopathy and their associated pathways could help researcher's to improve understanding of the pathway pathophysiology and its mechanism of action. As per earlier studies conducted by Barba et al. (2010) confers the existence of metabolite markers of diabetic retinopathy in the vitreous humor. However, the invasiveness of vitreoussampling limits the potential for study replication andclinical translation of any biomarkers recognized from vitreousfluid. Contrary to this sampling units, serum or plasma fluids remains the choice of metabolic fluids (Chen et al. 2016).

Current study aimed to investigate the plasma metabolic profiling of proliferative diabetic retinopathy and to detectassociated metabolite markers.

The investigation ofconcerning metabolite markers will be helpful to examined extensively theirmechanism of the occurrence and progression of retinopathy in diabetic patients atdifferent stages of disease.

2. Materials Methods

2.1 Sample selection

Subjects with eye defects along with type-II diabetes from our prior cohort studies (Yang et al. 2017) executed a case-control study to investigate a metabolite profiling specific to retinal damage. Our study selected those patients who have more than 7.5 percent glycated hemoglobinA1c (HbA1c) count (58mmol/mol) diagnosed to screen-out eye phenotypic character. Clinically a comparative study was performed in between the control and case subjects, patients with sign of eyesight-threatening or with duration of disease existence is more than 10 years were treated as case subjects (proliferative diabetic retinopathy, PDR), but without any degree of DR were assigned as non-diabetic retinopathy (NDR), shown in fig.1.

2.2 Exclusion criteria

Those individuals with other severe complications such as severe visual impairment caused by other reasons or with abnormal functioning of kidney, liver and heart were not considered as subjects for further studies. All of the undertaken subjects have previous medical records and were undergone for a physical examinations including age, gender, study duration, body mass index (BMI) and blood pressure.Despite of these subjects tested for blood and urine tests that included triglycerides, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total cholesterol (TC), serum creatinine, HbA1c and fasting plasma glucose concentration. Blood sample was collected in K2-EDTA tubes and each tubes were centrifuge at 3000g for 10min at 4°C to separate plasma fraction from received whole blood sample.

Separated plasma fraction were stored at -80°Cas mentioned in fig.1. The present study was approved and followed all the norms and regulation before conduction of the study of the university.

2.3 Metabolites identification

Collected serum fraction of the type-II diabetic patients were analyzed and quantified the metabolites with the help of using the liquid chromatography mass spectrometry (LC-MS) containing flow-injector chamber assembly. Along with this absolute IDQ1-p180 kit (Sigma Aldrich) was used to perform metabolites identification for further study. A significant differences in the introduced groups in terms of metabolite concentration were analyzed.All studied protocols were executed as described previously (Yun et al. 2019; Lee et al. 2016). However, all the serum samples were examined using the API-4000QTRAP LC/MS/MS system (Applied Biosystems, Foster City, CA, USA) and the Shimadzu HPLC system (Tokyo, Japan). Calibration standards and three different quality controls were introduced usingthe AbsoluteIDQ1 p180 Kit to serve as references for calibrating the exact metabolite concentrations in serum samples. In addition normal human serum samples were considered as reference standards. Qualitative data of metabolite obtained from each experiment records and follows the subsequent criteria.

First, the coefficient of variation (CoVs) of the metabolites in 10 reference standards must bebelow 15%. Second criteria, 50% of the measured metabolite concentrations exists in both the reference andserum samples must be beyond the limit of detection that was set to three times the median value of the three blank samples in each plate. After the quality control procedures, the resultant

122 metabolites were support theabove-statedstandards, and finalized sample analysis was selected for further statistical analyses.

2.4 Statistical analysis

All the obtained data were presented as (Mean±SD), while continuous data were analyzed with student-t-test using the SPSS software (22.0 vers. Chicago, IL, USA). This statistical t-test was performed to compare the metabolite concentration in the serum of NDR, and PDR patients. A comparison is based on the odds ratio (OR) calibrated at 95% confidence interval (CI) (per metabolite) using a multiple logistic regression model to adjust for covariates such as age or gender. In addition multiple correlation of metabolites concentration with associated risk factors were studied by adjusting the p-value functions. LASSO-LR based machine learning model was also introduced to derive a PDR or NDR diagnosis risk score.

3. Results and Discussion

In the present study work, the studied population was consists of (T2D) type-II diabetes (n=317)comprising of NDR (n=141) and DR (n=176) as shown in table.1. Diabetic group patients were further sub grouped into two groups based on the existing complications. It include the category of non-proliferative diabetic retinopathy (NPDR) with (n = 123) and proliferative diabetic retinopathy (PDR) with (n = 51) groups. Average T2D patient's duration of each group was 14.9years for NPDR, 20 years for PDR and 7.1 years NDR. Although, creatinine and HbA1c level was observed to be significantly differed among the studied groups.

Table.1 Biochemical and clinical analysis of studied groups

Parameters	NDR (n=141)	DR (n=176)	p-value (NDR vs DR)	(NPDR) (n = 123)	p-value (NDR vs NPDR)	(PDR) (n = 51)	p-value (NDR vs PDR)	p-value (NPDR vs PDR)
Female (%)	37.8	41.9	0.4494	39.8	0.73	47.1	< 0.01	0.3885
Age (years)	54.89 (11.34)	62.18 (11.66)	< 0.01	62.60 (11.60)	< 0.01	61.18 (11.87)	0.257	0.4698
Height (cm)	164.29 (8.58)	162.95 (8.59)	0.1878	162.94 (9.14)	62.48 (10.89)	1162.97 (7.22)	0.3061	0.9809
Weight (kg)	66 (10.76)	65.95 (10.12)	0.5602	66.03 (10.43)	164.03 (7.09)	64.99 (9.38)	0.3771	0.5272
BMI (kg/m^2)	24.53 (3.38)	24.76 (3.41)	24.89 (3.56)	24.89 (3.56)	67.17 (8.21)	24.48 (3.06)	0.919	0.6432
HbA1c (%)	7.38 (1.84)	8.19 (1.89)	< 0.01	8.07 (1.78)	< 0.01	8.49 (2.14)	< 0.01	0.2363

Creatinine (mg/dL)	0.96 (0.18)	1.21 (0.58)	< 0.01	1.10 (0.36)	< 0.01	1.49 (0.87)	< 0.01	< 0.01
Glucose (mg/dL)	148.20 (59.58)	173.52 (77.41)	< 0.01	172.98 (71.65)	< 0.01	174.82 (90.61)	0.05955	0.8999
Duration of T2D	7.1 (4.9) -	16.43 (11.1)	-	14.9 (8.2)		20 (15.48)	-	-

However, glucose levels and age of two studied groups was differed when compared to NDR versus DR and NDR versus NPDR, except NDR versus PDR. Whereas, gender was found to be significantly differed only in the comparative analysis of NDR versus PDR studied group. Remaining factors such as height, weight, and BMI do not show any significant differences in the three examined groups. Thus age factor was used as a covariate for groups; NDR versus DR and NDR versus NPDR, whereas gender was used as a covariate for NDR versus PDR group.

3.1 Metabolites detection in NDR and DR groups

Detection of metabolites in the serum of NDR and DR groups in terms of concentration differences and it was analyzed using a multiple logistic regression analysis where DR was treated as a dependent variable and each metabolite was consider as an explanatory variable, with adjustment for age.

Regression analysis was performed to find the concentrations of existing sixty two metabolites in the two analyzed groups, it concentration was differed significantly at (P <0.05) as shown in table.1. Current study results shows various kind of metabolites among them two metabolites are acylcarnitines (propionylcarnitine (C3) and butyrylcarnitine (C4), two biogenic amines (creatinine and total dimethylarginine (DMA), hexose, and one amino acid (proline), showed significantly higher in concentrations in the DR group as compared to NDR groups. As per previously encoded by Malecki et al. (2007) and Opatrilova et al. (2018) stated that the presence of total DMA in higher concentration in the plasma of DR concluded that increase the chances of retinopathy complications in T2D patients as these complications are associated with Asymmetric DMA level (ADMA). Increased ADMA level inhibits the activity of nitric oxide synthetase (NOS), which reduces the level of nitricoxide (NO) which in turn led to such kind of diabetic complications. Other studies Raddino et al. (2007) also supported the current study results and reported that increased ADMA level is linked to the NO in DR and even their shortage increases the chances of cardiovascular risk.Consequently, it was revealed that higher circulating plasma ADMA level is associated with DR in T2D. Based on the results of earlier studies and of this study results depicted that total DMA and ADMA may be indicative metabolites of DR, associated with the regulation of NO synthesis in the blood of T2D patients.

3.2 Detection of the common metabolites with differences in their concentration in the NPDR and NPR patients, comparative analysis with the NDR patients

The concentrationdifference in common metabolites was found to be significant in theirtwo studied groups, *i.e.*NDR versus NPDR and NDR versus PDR, the same statistical analysis was done for each group. Then, the common metabolites of the groups in each studied group were selected. Fifty-three metabolites exhibit different concentrations in both NDR and NPDR groups (Table 2). However, three amino acids (alanine, aspartic acid, and glutamine), total DMA, and hexose showed significant concentration in the NPDR patients as compared to NDR patients. Three derivatives of carnitines were exists as acylcarnitines (carnitine [Co], tetradecenoylcarnitine [C14:1], and hexadecanoylcarnitine[C16]), and seven other amino acids such as arginine, histidine, lysine, methionine, threonine, tryptophan, and tyrosine are also found.

Along with this, thirty-eight glycerophospholipidsdisplayedlesser concentrations in the NPDR patients over NDR patients. Existing 30 metabolites had significant differences in the concentration in the PDR than in the NDR subjects.

Other than this, three short chain namely acylcarnitines (C3, C4, and valerylcarnitine [C5]) and three biogenic amines (creatinine, kynurenine, and total DMA) were exists in a higher concentration in the PDR subjects rather than NDR subjects.Furthermore,Fiedorowicz et al. (2019) report illustrated that the role of the tryptophan-kynurenine metabolism pathway has been studied in animal models and human individuals with retinal and optic nerve damage, but not found any role in diabetic retinopathy because metabolism of tryptophan produces kynurenine as metabolic product, and its concentration was decreased in the animal and human models. The concentration of kynurenine, a product of tryptophan metabolism, was reciprocally increased as the activity of indoleamine, 2,2dioxygenase is elevated as observed in PDR vs NPDR patients.However no significant change in tryptophan level was observed but this study found that tryptophan level was decreased in both NPDR and PDR patients than in the NDR (table 2). Moreover, the kynurenine level was found significantly greater in PDR versus NDR patients (Odd ratio: 1.75). And same thing is applicable in PDR versus NPDR patients, and in DR versus NDR patients; however, these differences showed no significant differences. Altogether, the results of this study further supportive to the investigation of tryptophan and kynurenine for the treatment of retinopathy in T2D patients. This study results concerning with serum creatinine was found in a significant amount in PDR patients compared to NDR and NPDR patients and this increased level could be a one of the reason of diabetic complications associated with renal dysfunctions in diabetic retinopathy (DR) patients. This study results were found consistent with the previously encoded study results as recorded by Hsieh et al. (2018).

However, this study noted the arginine concentration was not significantly altered in the DR patients, than in the NDR patients except carnitine, C7: DC, it was significantly increased in the PDR patients than NPDR patients. Furthermore, other carnitines levels such as C14:1 and C16, were decreased in the DR (NPDR and PDR) patients over NDR patients. Since our results were founddistinct from previous studies encoded by Sumarriva et al. (2019), included many carnitine subtypes, the levels of each carnitines in encoded study result did not found compatible with the other earlier studies.Haines et al. (2018) analysis with differed sample sources *i.e.* serum and

vitreous humor specify the presence of six common metabolites including proline, citrulline, kynurenine, creatinine, glutamine, and methionine in serum samples, that's why;it could be used for detection of DR-specific metabolites, even though vitreous humor sample showed the actual status of DR.For DR specific metabolites detection purposes only DR serum samples are recommended excluding samples collected from nephropathicprone patients or taken from diabetic patients and found compactible with other reported previous studies.

Conversely, our results showed that there was presence of five long-chain fatty acids such asacylcarnitines (C14:1, C16, octadecanoylcarnitine [C18], octadecenoylcarnitine [C18:1], and octadecadienylcarnitine [C18:2]), thirteen glycerophospholipids, and five other amino acidsnamely lysine, methionine, serine, tryptophan, and tyrosine but in lower concentrations especially in the PDR patients than in the NDR patients.Instead of this, the present study showed the presence of existing 16 identical metabolites in a significantamount but differed concentrations in both the NPDR andPDR patientscomparative to the NDR patients, therefore it was concluded that finally selected metabolites are specific type as presented in(Table 2). Among them, only total DMA had significantly higher concentrations in thePDR and NPDR patients compared to NDR patients, while the other additional metabolites were found in a lowerconcentrations in both PDR and NPDR groups, compared to the NDR groups.

Table 2. Common metabolites detection in non-proliferative (NPDR) and proliferative diabetic retinopathy (PDR) disease stage

Metabolites	Logistic regression		Annova analysis	
	Odds ratio	p-value	Fold change	p-values
Tetradecenoylcarnitine (C14:1)	0.63 (0.48–0.82)	4.32E-03	0.87	3.51E-04
Hexadecanoylcarnitine (C16)	0.59 (0.45–0.75)	6.68E-04	0.86	1.95E-05
Lysine (Lys)	0.63 (0.49–0.81)	2.90E-03	0.92	2.15E-04
Methionine (Met)	0.53 (0.4–0.69)	1.69E-04	0.88	9.26E-07
Tryptophan (Trp)	0.36 (0.26–0.49)	4.48E-08	0.81	1.79E-12
Tyrosine (Tyr)	0.43 (0.31–0.57)	1.42E-06	0.84	4.84E-10
Total Dimethyarginine (Total DMA)	2.3 (1.59–3.47)	4.28E-04	1.31	4.73E-05
Phosphatidylcholinediacyl C32:2 (PC aa C32:2)	0.47 (0.34–0.62)	1.82E-05	0.75	4.79E-08
Phosphatidylcholinediacyl C34:2 (PC aa C34:2)	0.56 (0.42–0.73)	6.68E-04	0.85	2.24E-05
Phosphatidylcholinediacyl C36:2 (PC aa C36:2)	0.56 (0.43–0.73)	4.28E-04	0.85	1.10E-05
Phosphatidylcholinediacyl C38:6 (PC aa C38:6)	0.61 (0.47–0.78)	1.49E-03	0.86	8.06E-05
Phosphatidylcholinediacyl C40:6 (PC aa C40:6)	0.6 (0.46–0.77)	1.15E-03	0.86	5.91E-05
Phosphatidylcholine acyl-alkyl C36:5 (PC ae C36:5)	0.67 (0.51–0.86)	1.03E-02	0.87	1.46E-03
Phosphatidylcholine acyl-alkyl C42:3 (PC ae C42:3)	0.69 (0.53–0.88)	1.60E-02	0.9	2.30E-03
Hydroxysphingomyeline C22:1 (SM (OH) C22:1)	0.6 (0.46–0.77)	1.22E-03	0.88	6.09E-05
Sphingomyeline C24:0 (SM C24:0)	0.57 (0.44–0.74)	4.28E-04	0.88	7.84E-06

3.3 Metabolites detection with concentration differences in the PDR and NPDR Subjects

Results of the study finds that there was presence of 8 metabolites in a significant amount but in different concentrations in PDR and NPDR patients (Table 2). Among them, 4 other identified metabolites were (pimelycarnitine [C7:DC], creatinine, totalDMA, and phosphatidylcholine (PC)aa(C32:2) and all existing metabolites are present in higher concentrations in the PDRpatients than in the NPDR patients. Rest other four identified metabolites were lyso PC a C18:2, PC aa C36:1,PC aa C44:4, and PC aa C44:5, and remaining four other metabolites were exists in lower concentrations in the PDR patients than in theNPDR patients.However, several studies

significantly showed the presence of phosphatidylcholine at different levels in the NDR and DR patients as this study result was found consistent with present study result.Basically phosphatidylcholinerole in retinopthay cases is not so well defined because not so much scientific information is available in human metabolite databases. However, the PCs detection were concerned to DR as metabolites in our study, as it will allow future studies to be conducted to understand the roles of PCs in DR disease progression in T2D patients.

Besides this, it is a cross-sectional study, where different metabolites were measured in a different proportion at a particular time. Furthermore, several otheranalytic metabolites indicative for DR progression in T2D patients were identified in this study that have been already detected in earlier studies. Of these identified metabolites total DMA, tryptophan, kynurenine, several carnitines, and creatinine, were showed the significant amount at different levels in the DR patients as compared to the NDR patients. It could be postulated that high serum creatinine and glucose levels in the DR patients might have significant affect on the differedconcentrations of existing metabolites in the DR patients compared to the NDR patients. From this experimental study it was concluded that authentication of experimental procedures is done in more sophisticated and considerable way as it is depend on the choice ofsamples selected or suitable animal models use for functional studies that will be helpful for the identification of other important metabolites concerning with DR progression in T2D patients.

4. Conclusion

From this study it was concluded that comprehensive metabolomics profiling associated with diabetic retinopathy in T-2 diabetic patients were studied. These novel DR-associated metabolites should be considered in the study, astheir detection is helpful to understand the molecular mechanism behind the initiation and progression of DR in T2D patients.The metabolitesrecognized in this study will provide path tounderstandmechanisms concerning to DR development and progression in T2D patients, as it is helpful to diagnosis disease development at early stages and provide a valuable insight to develop appropriate therapeutic measures.

5. References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. (2017). IDF Diabetes Atlas: Global Estimates for the Prevalence of Diabetes for 2015 and 2040. Diabetes Res ClinPract128:40-50.
2. Sumarriva K, Uppal K, Ma C, Herren DJ, Wang Y, Chocron IM, et al. Arginine and Carnitine Metabolites Are Altered in Diabetic Retinopathy. Invest Ophthalmol Vis Sci. 2019; 60(8):3119–26.
3. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. (2017). Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. JAMA. 317:2515–23.
4. Ting DS, Cheung GC, Wong TY. (2016). Diabetic retinopathy: global prevalence, major risk factors, screening practices and public health challenges: a review. Clin. Exp. Ophthalmol. 44:260-77.

5. Lachin JM, Genuth S, Nathan DM, Zinman B, Rutledge BN, (2008). Group DER. Effect of glycemic exposure on the risk of microvascular complications in the diabetes control and complications trial-revisited. Diabetes. 57:995-1001.
6. Chen L, Cheng CY, Choi H, Ikram MK, Sabanayagam C, Tan GS, Tian D, Zhang L, Venkatesan G, Tai ES, et al. (2016). Plasma Metabonomic profiling of diabetic retinopathy. Diabetes. 65:1099-108.
7. Pirola L, Balcerczyk A, Okabe J, El-Osta A. (2010). Epigenetic phenomena linked to diabetic complications. Nat. Rev.Endocrinol.6:665-75.
8. Nunes S, Ribeiro L, Lobo C, Cunha-Vaz J. (2013). Three different phenotypes of mild non-proliferative diabetic retinopathy with different risks for development of clinically significant macular edema. Invest Ophthalmol. Vis. Sci. 54:4595-604.
9. Tan GS, Ikram MK, Wong TY. (2013). Traditional and novel risk factors of diabetic retinopathy and research challenges. Curr. Med. Chem. 20:3189-99.
10. Holmes E, Wilson ID, Nicholson JK. (2008). Metabolic phenotyping in health and disease. Cell. 134:714-7.
11. Kuo JZ, Wong TY, Rotter JI. (2014). Challenges in elucidating the genetics of diabetic retinopathy. JAMA Ophthalmol. 132:96-107.
12. Barba I, Garcia-Ramirez M, Hernandez C, Alonso MA, Masmiquel L, Garcia- Dorado D, Simo R. (2010). Metabolic fingerprints of proliferative diabetic retinopathy: an 1H-NMR-based metabonomic approach using vitreous humor. Invest Ophthalmol Vis Sci. 51:4416–21.
13. Yang JK, Wang YY, Liu C, Shi TT, Lu J, Cao X, Yang FY, Feng JP, Chen C, Ji LN, Xu A. (2017). Urine proteome specific for eye damage can predict kidney damage in patients with type-2 Diabetes: a case-control and a 5.3-year prospective cohort study. Diabetes Care. 40:253-60.
14. Yun JH, Lee HS, Yu HY, Kim YJ, Jeon HJ, Oh T, et al. (2019). Metabolomics profiles associated with HbA1c levels in patients with type 2 diabetes. PLoS One. 14(11):e0224274.
15. Lee H-S, Xu T, Lee Y, Kim N-H, Kim Y-J, Kim J-M, et al. (2016). Identification of putative biomarkers for type 2 diabetes using metabolomics in the Korea Association REsource (KARE) cohort. Metabolomics. 12(12).
16. Malecki MT, Undas A, Cyganek K, Mirkiewicz-Sieradzka B, Wolkow P, Osmenda G, et al. (2007). Plasma asymmetric dimethylarginine (ADMA) is associated with retinopathy in type 2 diabetes. Diabetes Care. 30(11):2899-901.
17. Opatrilova R, Kubatka P, Caprnda M, Busselberg D, Krasnik V, Vesely P, et al. (2018). Nitric oxide in the pathophysiology of retinopathy: evidences from preclinical and clinical researches. ActaOphthalmol. 96(3):222-31.
18. Raddino R, Caretta G, Teli M, Bonadei I, Robba D, Zanini G, et al.(2007). Nitric oxide and cardiovascular risk factors. Heart Int. 3(1):18.
19. Fiedorowicz M, Choragiewicz T, Thaler S, Schuettauf F, Nowakowska D, Wojtunik K, et al. (2019).Tryptophan and Kynurenine Pathway Metabolites in Animal Models of Retinal and Optic Nerve Damage: Different Dynamics of Changes. Front Physiol.10:1254.

20. Hsieh YT, Tsai MJ, Tu ST, Hsieh MC.(2018). Association of Abnormal Renal Profiles and Proliferative Diabetic Retinopathy and Diabetic Macular Edema in an Asian Population with Type 2-Diabetes. JAMA Ophthalmol. 136(1):68-74.
21. Haines NR, Manoharan N, Olson JL, D'Alessandro A, Reisz JA. (2018). Metabolomics Analysis of Human Vitreous in Diabetic Retinopathy and Rhegmatogenous Retinal Detachment. J Proteome Res. 17 (7):2421-7.