Association of FBN1 genetic variant(rs370096856) with serum Asprosin level among Type 2 Diabetes Mellitus Subjects-A Cross Sectional Study

Mary Yanglem[1], Shetty Sukanya[1*], Bhandary Roopa[1], Chakraborty Gunimala[2] and Kulkarni Vineet[3]
1. Department of Biochemistry, K.S. Hegde Medical Academy, Nitte (Deemed to be University) Mangaluru, INDIA
2. Department of Molecular Genetics and Cancer, Nitte University Centre for Science Education and Research, Nitte (Deemed to be University, Mangaluru, INDIA
3. Department of Biochemistry, Tejaswini Institute of Allied Health Science, Mangaluru, INDIA
*shettysukan@nitte.edu.in

Abstract

The global burden of Type 2 diabetes mellitus (T2DM) continues to rise even with advancement in therapeutic options. There is a pressing need for new biomarkers to improve prognosis. Recent epidemiological and genetic studies have emphasized the crucial role of serum asprosin levels in insulin resistance and diabetes complications. However, the role of genetic variants in serum asprosin levels remains poorly understood. Fifty subjects with T2DM who met the inclusion criteria were recruited for the study. Serum asprosin levels were estimated using the ELISA method. SNP genotype analysis for rs370096856 was conducted using PCR and Sanger sequencing.

The present study demonstrated that serum asprosin levels were significantly elevated (p=0.012) in subjects with the GT genotype compared to those with GG and TT genotypes. Among subjects with the GT genotype, asprosin levels showed a positive correlation with TyG (r=0.6), LDL (r=0.78) and HbA1c (r=0.08). The ROC analysis revealed the cut-off value of 2.9 ng/mL asprosin to predict the risk of T2DM.The present study highlighted a positive correlation between asprosin and TyG among subjects with GT genotype. Further multicentric studies with larger sample sizes are necessary to confirm these findings.

Keywords: Asprosin, single nucleotide polymorphism, type 2 Diabetes Mellitus.

Introduction

Type 2 Diabetes Mellitus(T2DM) is a worldwide public health burden, which accounts for about 90–95% of diabetes cases. Over the past 40 years, the number of adults with T2DM has quadrupled from 108 million in 1980 to 463 million in 2019 globally[6,20]. This figure is predicted to be 579 million in 2030 and 700 million in 2045[3]. The aetiology of T2DM is multifaceted and has not yet been fully elucidated. T2DM is characterised by defective insulin secretion and reduced sensitivity to it, leading to chronic hyperglycaemia and severe metabolic dysfunction in patients[19]. Hyperglycaemia affects the physiological function of several tissues and organs in the body, among which the most common are neuropathy and vascular complications[12].

Although several novel biomarkers such as glycated albumin, 5-anhydroglucitol and lifestyle risk factors like obesity, alcoholism, physical inactivity were extensively studied and predictive values have been tested, most studies to date have only reported modest improvements in prediction of novel biomarkers over conventional biomarkers such as HbA1c[14,18].

Adipose tissue dysfunction results in altered adipokine secretion potentially contributing to various metabolic diseases, such as obesity, T2DM and cardiovascular disorders[16]. Asprosin, a novel adipokine is the C-terminal cleavage product of profibrillin encoded by the FBN1 gene. Asprosin is secreted by white adipose tissue in response to hypoglycemia. It has been reported that asprosin activates protein kinase A (PKA) in the liver, followed by the release of glucose from hepatocytes[9]. Clinical and preclinical studies have revealed altered levels of circulating asprosin in numerous metabolic disorders, such as polycystic ovarian syndrome (PCOS), obesity, non-alcoholic fatty liver disease (NAFLD), T2DM and various malignacies[16].

Few case-control studies have demonstrated a genetic predisposition to T2DM. Many SNPs related to the susceptibility to T2DM have been discovered by genome-wide association studies in different populations[21]. rs370096856 is missense SNP located in 65th exon of FBN1 gene. *In silico* analysis showed that rs370096856 is found to be up regulate asprosin protein expression, however association between rs370096856 and asprosin level among subjects with T2DM is least understood[11]. This cross sectional study was carried out to find association between FBN1 genetic variant (rs370096856) and serum asprosin level among subjects with T2DM.

Material and Methods

A total of 50 T2DM subjects who attended the outpatient and inpatient Department of Medicine of Justice K.S. Hegde Charitable Hospital between 1st April 2023 to 1st April 2024 were included for the study. This study was approved by the Institution Ethics Committee of K S Hegde Medical Academy, Mangaluru, Karnataka, India (Registration No.: EC/NEW/INST/2022/KA/0174, Date: 1st April 2023, File No.-INST.EC/ EC/049/2023). All participants provided

informed consent before participation. The study adhered to the principles of Declaration of Helsinki, 2013. Subjects aged above 18 years comprising of 29 males and 21 female diagnosed with T2DM as per the American diabetes association guidelines were included in the study. Subjects with Type 1 Diabetes, autoimmune disorders, cancers and any other severe infections that might cross react with variables under investigation were excluded from the study. Baseline characteristics such as age, gender and HbA1c along with biochemical parameters were obtained from subject case sheets. Body mass index (BMI) was derived from weight and height measurements. The triglyceride glucose index (TyG index) was calculated using the formula: TyG = Ln (Fasting triglycerides in mg/dL) * (Fasting plasma glucose in mg/dL) / 2.

4ml of blood samples was collected from the study subjects. Among these, 2ml was collected in a plain blood collection tube for asprosin analysis, while another 2ml was taken in an EDTA tube for DNA isolation. To obtain serum for asprosin analysis, the samples were centrifuged at 3000 rpm for 10 minutes and aliquots were stored at 4^0 C till analysis. The blood samples for DNA isolation and gene polymorphism were also stored at -20^0 C till analysis. The estimation of serum asprosin was performed using a standard Elisa kit from Krishgen Biosystem in Bangalore. The method employs sandwich Elisa technique.

Monoclonal antibodies were pre-coated into microwells. Samples and standards were pipetted into microwells and Human asprosin present in the sample was bound by the antibodies. Biotin labeled antibody was added and followed by Streptavidin. HRP was pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) was added to microwells and color develops proportionally to the amount of human asprosin. Color development was then stopped by addition of stop solution and absorbance was measured at 450 nm.

For the SNP genotype analysis, genomic DNA was isolated using the phenol-chloroform-isoamyl alcohol method. Genotyping of the FBN1 SNP (rs370096856) was determined through polymerase chain reaction (PCR). PCR reactions were performed in 15 µL volume reaction mixtures using about 1 µL (10 ng/ µL) of genomic DNA, 14 µL Master mix (2×) (Taqman, Thermo Fisher Scientific, USA), 6 µL of nuclease-free water (ddH2O), 1 µL of each primer. Products were amplified in Biorad T100 for 34 cycles under the following conditions: denaturing at 94°C for 5 min and 94°C for 30 seconds, annealing at 58°C for 30 s. The final extension was extension at 72°C for 5 minutes. The PCR products were separated using a 2% agarose gel and the results were visualized under a UV trans-illuminator. Finally, all 50 samples were sent to a commercial vendor for Sanger sequencing.

Statistical analysis: Statistical analysis was performed using SPSS version 21. Continuous variables like age, BMI, lipid profile, HbA1c and asprosin were presented as mean±S.D and compared using one way ANOVA. Categorical variables such as gender and genotype were represented as frequency and percentage and compared using chi square test. Correlation between Asprosin and lipid profile was determined using Spearman's correlation analysis. SNP was assessed for Hardy-Weinberg equilibrium (HWE) and considered a *p* value less than 0.05 as statistically significant.

Results and Discussion

A total of 50 study participants with T2DM were enrolled in this study. Among study subjects, male was predominant (N=29) when compared to female (N=21). Further T2DM subjects were sub divided into three groups based on Sanger's sequencing results (GG, GT, TT) (Fig. 3). The subjects with GT genotype(N=24) were more than those with GG (N=15) and TT genotype (N=11) (Table 2). No significant difference was observed in age(p=0.7), BMI (p=0.5) and gender(p=0.9) among three groups. Subjects with GT genotype had elevated FPG level when compared to others, though it was not statistically significant.TG level was significantly higher among GT subjects in comparison with GG and TT(p=0.041). In T2DM patients, a high level of TC, LDL, HDL, TYG, TG/HDL, LDL/HDL was found to be increased in GT genotype than others. However, these findings were not statistically significant (Table 2).

Asprosin and HbA1c levels were analysed among T2DM subjects with GG, GT and TT genotype. Present study showed that both asprosin (p-0.012) and HbA1C(p-0.014) level were significantly elevated in subjects with GT genotype when compared to subjects with GG and TT genotype (Table 3) (Figs. 2, 3 and 4).

Asprosin was positively correlated with TyG(r-0.6), LDL(r-0.78) and HbA1c(r-0.08) among subjects with GT genotype. However, there was no positive correlation observed between asprosin and TC(r=0.13), FPG(r=0.18) and HDL(r=0.08) among subjects with GT genotype (Table 5).

Table 1
rs370096856 primer sequence

Oligo Name	Primer Sequence (5'-3')	Tm
Asprosin Forward	CCTGTCAGTGGTGTTCTTGG	63.7
Asprosin Reverse	TTCCACCACAGGAGACATC	64.4

Details of forward and reverse primers used for SNP analysis. Tm - melting temperature, SNP-Single nucleotide polymorphism

Table 2
Biochemical parameters and baseline characteristics among study subjects

Parameter	GG(N=15)	TT(N=11)	GT(N=24)	p value <0.05
AGE(years)	59.3±9.3	58.3±9.2	59.7±9.3	0.7
BMI(kg/m^2)	24±2.3	23.2±5.3	24.6±3.1	0.5
GENDER				
Male(N)	9	6	14	0.9
Female(N)	6	5	10	
FPG(mg/dL)	161±54	151±43	174±61	0.6
TG(mg/dL)	138±34	192±51	145±67	0.041*
TC(mg/dL)	147±33	177±54	184±57	0.3
LDL(mg/dL)	89±32.1	121±35	128±55	0.1
HDL(mg/dL)	39±9.8	38±8.4	40±7.6	0.7
Non HDL (mg/dL)	108	139	144	0.04
TYG	9.3±0.4	9.1±0.6	9.4±0.6	0.5
TG/HDL	3.8±1.6	5.1±2.7	3.8±1	0.1
LDL/HDL	2.3±1	3.2±0.9	32±1.4	0.3

Table showing biochemical parameters and baseline characteristics among study subjects. Continuous data such as age,BMI, FPG, lipid profile were represented as mean±S.D. Categorical data like gender was represented as frequency. BMI- body mass index FPG- fasting plasma glucose, TG- triglyceride, TC-total cholesterol, LDL-low density lipoprotein, HDL- high density lipoprotein, TYG- triglyceride glucose index.

Table 3
Asprosin and HbA1c among study subjects.

Parameter	Total	GG(N=15)	TT(N=11)	GT(N=24)	p value <0.05
Asprosin (ng/ml)	4.86±2.1	3.9±2.3	4.5±3.5	6.18±1.9	0.012*
HbA1c (%)	7.06±1.5	6.3±0.73	6.9±1.18	8±2.6	0.014*

Table showing Asprosin and HbA1c data among study subjects. Asprosin data such as wasrepresented as mean±S.D. HbA1c-Glycated hemoglobin

Table 4
Correlation analysis of Asprosin with Biochemical parameters among subjects with GTgenotype

Biochemical parameters	r value	p value <0.05
TC	0.13	0.6
TYG	0.6	0.02*
LDL	0.78	0.009**
FPG	0.18	0.53
HDL	0.08	0.12
HbA1C	0.8	0.041*

Correlation analysis was performed using Pearson's correlation method. TC-total cholesterol, LDL- low density lipoprotein, HDL-high density lipoprotein, TYG- triglyceride glucose index, FPG- fasting plasma glucose

Table 5
Correlation analysis of Asprosin with Biochemical parameters among subjects with GG genotype.

Biochemical parameters	r value	p value <0.05
TC	0.9	0.001**
TYG	0.7	0.03*
LDL	0.03	0.12
FPG	0.6	0.02*
HDL	0.4	0.09
HbA1c	0.25	0.15

Correlation analysis was performed using Pearson's correlation method. TC-total cholesterol, LDL- low density lipoprotein, HDL-high density lipoprotein, TYG- triglyceride glucose index, FPG- fasting plasma glucose.

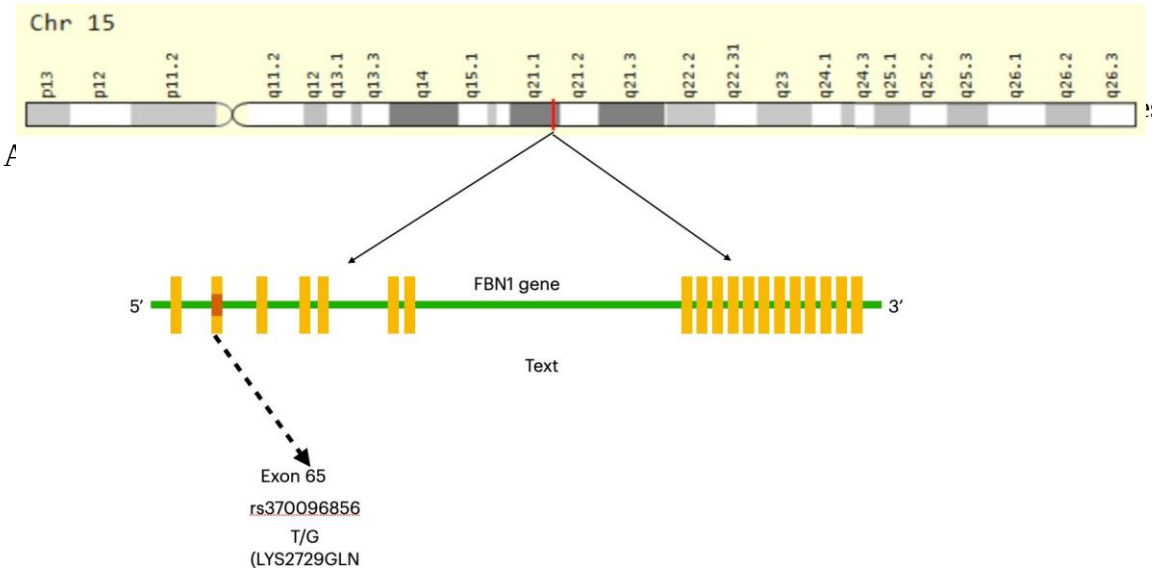

Fig. 1: Location of SNP in FBN1. The figure showing location of SNP in exotic region of FBN 1 gene present in chromosome number 15(GeneCARD)

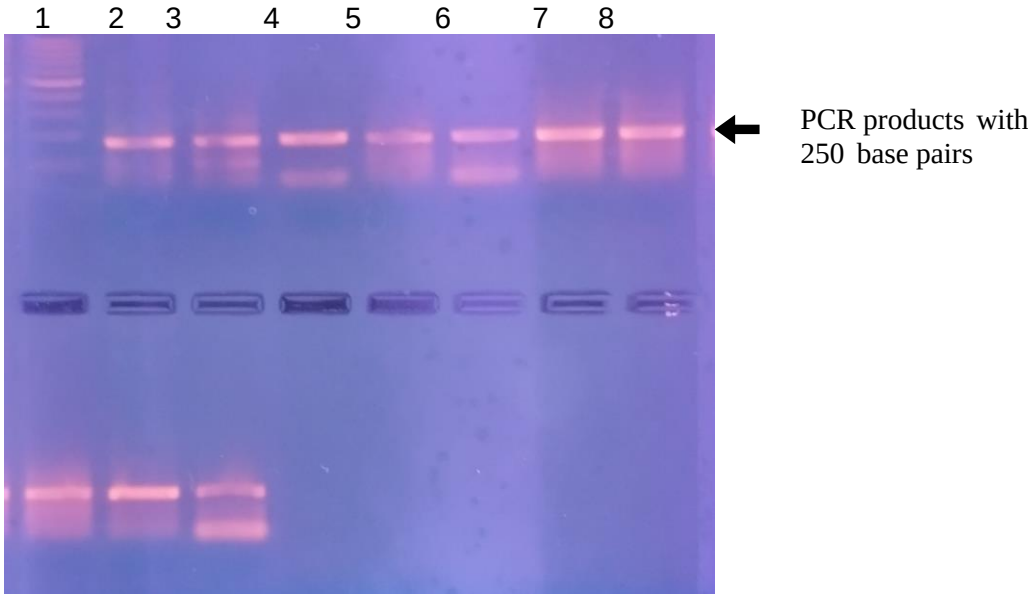

Fig. 2: 2 % Agarose Gel electrophoresis showing PCR amplicons products for SNP (rs370096856).
Lane 1 representing DNA ladder of 100 base pairs.
Lane 2-8 representing genomic DNA with 250 base pair size

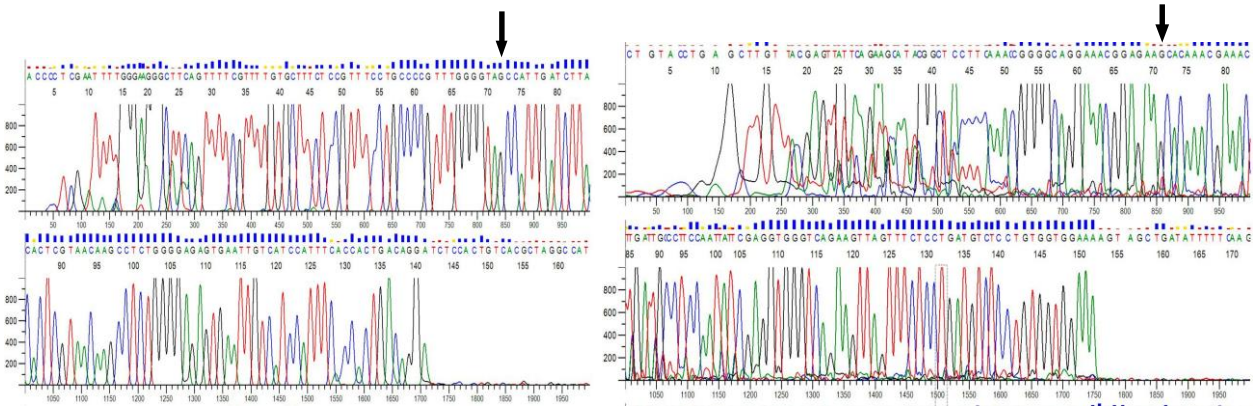

Fig. 3: Representative Sanger's sequencing image showing homozygous and heterozygous allele for SNPrs370096856) at 73rd position where C is replaced by G

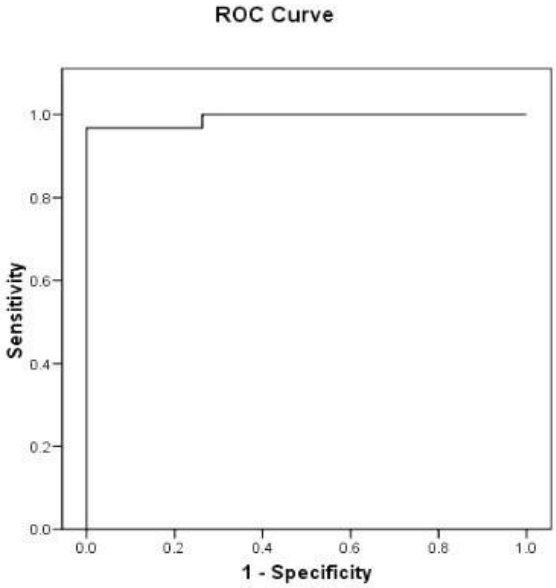

Fig. 4: Receiver Operator Curve(ROC) showed that cut off value for asprosin was found to be 2.9ng/ml. Area under curve-0.992

Subjects with GG genotype showed positive correlation between asprosin and TC (r-0.9), TyG(r=0.7) and FPG(r=0.02) whereas there was no correlation observed between asprosin and LDL(r=0.03), HDL(r=0.4), HbA1C(r=0.25) (Table 5).

The ROC analysis for serum Asprosin level, among T2DM subjects with GT, GG and TT genotype revealed the area under the curve (AUC) of serum Asprosin (ng/mL) as 0.992 with confidence interval (95% CI). The threshold value for Asprosin was found to be 2.9 ng/mL (Fig. 4). T2DM comprises of 90–95% of all diabetes cases[17]. It frequently remains undiagnosed for several years because high blood sugar levels develop gradually and may not initially cause noticeable symptoms such as dehydration or unintentional weight loss[4]. Nonetheless, undiagnosed individuals with T2DM are at a heightened risk of developing significant complications[5]. It is often strongly linked to genetic predisposition or family history in first-degree relatives. Nevertheless, the genetics of type 2 diabetes is currently poorly understood and is under intensive investigation in the era of precision medicine[23].

The discovery of Asprosin opened up new pathways for understanding the impact of it on metabolic disorders such as T2DM, obesity, polycystic ovary syndrome, gestational diabetes mellitus and cardiometabolic diseases. Asprosin, a gluconeogenic and prophagocytic adipokine primarily released by white adipose tissue, affects various organs and tissues in the body. It has been observed to influence the heart, liver, pancreas and skeletal muscle through proinflammatory responses, oxidative stress, endoplasmic reticulum stress and apoptosis[24].

As a C-terminal cleavage product of fibrillin 1, asprosin is encoded by the FBN1 gene located at the 15q21.1 chromosomal region[10]. While FBN1 variants have been associated with diverse clinical phenotypes, there is still much to learn about the relationship between different FBN1 regions and specific phenotypes[2].

While our understanding of FBN1 variants and their implications is incomplete, the discovery of asprosin has provided valuable insights into the complex mechanisms at play in various metabolic disorders. The aim of this cross-sectional study was to explore the association between FBN1 SNP rs370096856 and asprosin level among T2DM subjects.

Results from the present study revealed that all biochemical parameters except TG were normal among T2DM subjects. This might be because most subjects were on lipid-lowering and oral hypoglycaemic medications during the recruitment period. In the present study, the mean serum asprosin was 4.9ng/mL. These findings follow the survey conducted by Alan et al[2] among the Turkish population. Asprosin level was found to be 5.05ng/mL[24]. Another study by Cui et al[7] reported that the mean asprosin level was 0.25ng/mL among Chinese subjects[2]. This discrepancy in asprosin level might be due to different study populations. The function of asprosin under physiological conditions, although not completely understood, is opposite to that of insulin, with low glucose levels (fasting) stimulating asprosin production and high levels (feeding condition) inhibiting it[7].

The present study reported that asprosin and HbA1c were significantly increased among subjects with the GT genotype. In addition, a significant correlation was observed between asprosin and HbA1C among GT genotypes for SNP rs370096856. This SNP is located at the 65th exon of the FBN1 gene. The role of this SNP in asprosin protein expression is yet to be investigated[13]. The cutoff value for asprosin to predict the risk of T2DM was found to be 2.9ng/dL(AUC-0.992) from ROC analysis. There are few

studies that reported higher asprosin cutoff values than the present study. The study conducted by Goodarzi et al[10] showed the asprosin cutoff value among T2DM subjects as 5.46ng/ml (AUC- 0.828). Deng et al[8] reported an asprosin cutoff value of 1.9ng/mL (AUC-0.623), less than the present study.

Conclusion

In conclusion, our study highlighted that serum asprosin and HbA1C significantly increased in T2DM subjects with GT genotype. Asprosin positively correlated with HbA1C among subjects with GT genotype. This study has certain limitations. First, this cross-sectional study assessed asprosin in a nonuniform manner at different points; thus, we did not discuss dynamic changes in serum asprosin level. Secondly, causal relationship between serum asprosin and T2DM needs to be confirmed. Lastly, physical activity and medication were not evaluated in this study.

Acknowledgement

The authors express deep gratitude for the resources and support offered by DST-FIST and NITTE (Deemed to be the University) for this study. Authors appreciate necessary guidance given by Dr. Anirban, Director and Dean, Faculty of Biological Sciences, Nitte university centre for science education and research (NUSCER).

References

1. Ahmad E., Lim S., Lamptey R., Webb D.R. and Davies M.J., Type 2 diabetes, *Lancet*, **400(10365)**, 1803–1820 **(2022)**

2. Alan M., Gurlek B., Yilmaz A., Aksit M., Aslanipour B., Gulhan I., Mehmet C. and Taner C.E., Asprosin: a novel peptide hormone related to insulin resistance in women with polycystic ovary syndrome, *Gynecol Endocrinol*, **35(3)**, 220-223 **(2019)**

3. Alrikabi R.M. and Ali H., Potential Protective Effect of Anti-zinc Transporter 8 Autoantibodies on Gestational Diabetes Mellitus in Iraqi Population, *BBRJ*, **8(1)**, 100-107 **(2024)**

4. Brown S.D., Hedderson M.M., Zhu Y., Tsai A.L., Feng J. and Quesenberry C.P., Uptake of guideline-recommended postpartum diabetes screening among diverse women with gestational diabetes: associations with patient factors in an integrated health system in USA, *BMJ Open Diabetes Res Care*, **10(3)**, 271-278 **(2022)**

5. Cao L., An Y., Liu H., Jiang J., Liu W. and Zhou Y., Global epidemiology of type 2 diabetes in patients with NAFLD or MAFLD: a systematic review and meta-analysis, *BMC Med*, **22(1)**, 101-108 **(2024)**

6. Chahar, Deeksha S., Gyanendra K.S., Sangeeta S., Satyendra K. and Ahmad M.K., Unraveling Epigenetic Signatures for Early Detection of Diabetes Nephropathy in Type 2 Diabetes: A Case–Control Investigation, *BBRJ*, **8(1)**, 108-116 **(2024)**

7. Cui J., Liu Y., Li M., Yin J., Yang J. and Xu L., Association of serum asprosin with metabolic dysfunction-associated fatty liver disease in older adult type 2 diabetic patients: a cross-sectional study, *BMC Endocr Disord*, **24(1)**, 27-33 **(2024)**

8. Deng X., Zhao Z., Zhao L., Wang C., Li Y. and Cai Z., Association between circulating asprosin levels and carotid atherosclerotic plaque in patients with type 2 diabetes, *Clin Biochem*, **109(110)**, 44–50 **(2022)**

9. Farrag M., Ait E.D., González R.M., Cordero B.A., Ruiz F.C. and Capuozzo M., Asprosin in health and disease, a new glucose sensor with central and peripheral metabolic effects, *Front Endocrinol (Lausanne)*, **13**, 101-107 **(2023)**

10. Goodarzi G., Setayesh L., Fadaei R., Khamseh M.E., Aliakbari F. and Hosseini J., Circulating levels of asprosin and its association with insulin resistance and renal function in patients with type 2 diabetes mellitus and diabetic nephropathy, *Mol Biol Rep*, **48**, 5443–5450 **(2021)**

11. Huang J., Mendelian randomization indicates a causal contribution of type 2 diabetes to retinal vein occlusion, *Front Endocrinol (Lausanne)*, **8(14)**, 114-120 **(2023)**

12. Jog K.S., Eagappan S., Santharam R.K. and Subbiah S., Comparison of Novel Biomarkers of Insulin Resistance with Homeostasis Model Assessment of Insulin Resistance, Its Correlation to Metabolic Syndrome in South Indian Population and Proposition of Population Specific Cutoffs for These Indices, *Cureus*, **15(1)**, 351-356 **(2023)**

13. Kalantari S., Pourshams A., Roudi R., Zali H. and Bandehpour M., Identification of Potential Common Molecular Factors of Pancreatic Cancer and Diabetes Mellitus using Microarray Data Analysis Combined with Bioinformatics Techniques and Experimental Validation, *BBRJ*, **5(3)**, 286-294 **(2021)**

14. Karagiannidis E., Moysidis D.V., Papazoglou A.S., Panteris E., Deda O. and Stalikas N., Prognostic significance of metabolomic biomarkers in patients with diabetes mellitus and coronary artery disease, *Cardiovasc Diabetol*, **21(1)**, 70 **(2022)**

15. Ke F., Xue G., Jiang X., Li F., Lai F. and Zhang M., Combination of asprosin and adiponectin as a novel marker for diagnosing non-alcoholic fatty liver disease, *Cytokine*, **134**, 1551-1556 **(2020)**

16. Long J., Yang Z., Wang L., Han Y., Peng C. and Yan C., Metabolite biomarkers of type 2 diabetes mellitus and pre-diabetes: a systematic review and meta-analysis, *BMC Endocr Disord*, **20(1)**, 174 (**2020)**

17. Memon H., Abdulla F., Reljic T., Alnuaimi S., Serdarevic F. and Asimi Z.V., Effects of combined treatment of probiotics and metformin in management of type 2 diabetes: A systematic review and meta-analysis, *Diabetes Res Clin Pract*, **202**, 110-118 **(2023)**

18. Nanayakkara N., Curtis A.J., Heritier S., Gadowski, Pavkov M.E. and Kenealy T., Impact of age at type 2 diabetes mellitus diagnosis on mortality and vascular complications: systematic review and meta-analyses, *Diabetologia*, **64(2)**, 275-287 **(2021)**

19. Ogurtsova K. et al, IDF diabetes Atlas Global estimates of undiagnosed diabetes in adults for, *Diabetes Res Clin Pract*, **183**, 205-210 **(2022)**

20. Rai S., Rai T., Acharya S., Alva P. and Dsa J., Prevalence and Risk Factors for Hypogonadism in Male Patients with Type 2 Diabetes Mellitus, *BBRJ*, **7(2)**, 288-292 **(2023)**

21. Suder A., Makiel K., Targosz A., Maciejczyk M., Kosowski P. and Haim A., Exercise-induced effects on asprosin and indices of atherogenicity and insulin resistance in males with metabolic syndrome: a randomized controlled trial, *Sci Rep*, **14(1)**, 985 **(2024)**

22. Xu M., Zhang C., Zhang L., Qu H. and Wang Y., Plasma Asprosin Concentrations are Associated with Progression of Diabetic Kidney Disease, *Diabetes Metab Syndr Obes*, **17**, 2235-2242 **(2022)**

23. Yuan M., Li W., Zhu Y., Yu B. and Wu J., Asprosin: A Novel Player in Metabolic Diseases, *Front Endocrinol (Lausanne)*, **11**, 64 **(2020)**

24. Zhang Z., Zhu L., Wang Z., Hua N., Hu S. and Chen A., Can the new adipokine asprosin be a metabolic troublemaker for cardiovascular diseases? A state-of-the-art review, *Prog. Lipid Res*, **91**, 240-246 **(2023)**.

(Received 17th September 2024, accepted 19th October 2024)