

## RESEARCH PAPER

# Association of Afamin Concentration with Type 1 and Type 2 Diabetes Mellitus

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### ABSTRACT:

Afamin is a protein in humans that plays a major role in the regulation of glucose metabolism, insulin sensitivity, so it may be related to diabetes mellitus. The aim of this study to evaluate the afamin concentration level in diabetic patients. This study was a case-control study blood samples were collected from 130 patients of Diabetes mellitus (DM) which divided into two groups (65 types1 DM and 65 type 2 DM) from Laila Qasim center for diabetes in Erbil City. In addition 90 healthy volunteers used as a control. Serum was separated to determine afamin concentration. The results showed that the level of afamin in type 2 diabetic patients were significantly higher than those of healthy adults ( $75.09 \pm 10.09$  mg/L), and ( $65.62 \pm 7.18$  mg/L) respectively, No significant differences in the concentration of afamin were observed between control and type 1 DM were ( $58.47 \pm 8.13$ ), and ( $63.07 \pm 5.53$ ) respectively. Our finding showed that increase serum afamin concentration in type 2 diabetes patients can be used as a biomarker for predicting diabetes mellitus.

KEY WORDS: Serum afamin, type 1 DM, type 2 DM

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## 1.INTRODUCTION :

Afamin also named vitamin E binding protein is a protein in humans encoded by the *AFM* gene, which was founded in 1994 as the fourth constituent of the human albumin gene family which is comprised of four genes, and these four genes encode structurally-related serum transport proteins or binding protein. Afamin is an 87 kDa glycoprotein with five predicted N-glycosylation sites with 15% carbohydrate content and 55% amino acid sequence similarity to albumin. Human afamin gene (*AFM*) sequence size 1981 bp. *AFM* gene in humans has 15 exons and composed of 599 amino acids. Genomic location on chromosome 4q13-3 in humans is mainly expressed in the liver and secreted into the bloodstream (Lichenstein et al., 1994).

Afamin protein acts similarly to albumin as a transport protein for small hydrophobic molecules. A high-level amount of this protein found in plasma but circulating plasma afamin is primarily of hepatic origin; the brain, kidney, testes, and ovaries have been found as additional afamin-expressing tissues, body fluids, like follicular, cerebrospinal, and seminal fluid. A strong association between afamin protein levels in these fluids (Jerkovic et al., 2005).

Prevalence of diabetes have been steadily increasing worldwide according to this data in the years 2017, 463 million people had diabetes worldwide, , approximately 90% of diabetes were type 2 DM (Vos et al., 2012). Another study shows that the number of patients with diabetes mellitus globally has four times more than in the past three decades (Zheng et al., 2018)

Diabetes mellitus divided into two main types which are type1 diabetes (T1DM) and type2

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diabetes (T2DM) the major difference between these two types are relate following criteria first of all age, amount of loss of Beta Cell function, and insulin resistance or associated autoantibodies. Also, there are several causes for diabetes classification which are obesity at a young age and genetic factors for T1DM and T2DM(Leslie et al., 2016)

T1 DM named insulin-dependent diabetes mellitus (IDDM), this name come from that the pancreas cannot produce enough insulin as a result of a loss of B cell, T1DM commonly known in children and early age population.

T2DM named non-insulin-dependent diabetes mellitus (NIDDM) because cells fail to respond to the insulin produced by B cell properly means cells of the body resist to insulin, therefore, named insulin resistance, T2DM more common in adult and old age persons but causes due to excessive body weight, lifestyle, healthy diet and insufficient exercise (Delhi, 2012).

The study results show that the strong relationship between afamin plasma levels and all the components of metabolic syndrome were shown to occur not only at baseline, but also at follow-up, these findings of hyperlipidemia, hyperglycemia, and obese phenotype suggest a possible role of afamin protein infrequently observed metabolic disturbances, such as metabolic syndrome (Kronenberg et al., 2014).

Another study concluded that the value of afamin plasma levels in the female with polycystic ovary disorder (PCOS), a disease directly associated with the related signs of metabolic syndrome and type 2 diabetes mellitus, regarding the presence of insulin resistance. Furthermore, women with insulin resistance exhibited higher levels of afamin, regardless of the presence of PCOS. The researchers concluded that afamin serves as a prognostic marker of insulin resistance in young women with PCOS (Seeber et al., 2014).

The data of a pooled study showed that more than 20,000 participants, according to this study afamin protein level can be used or having benefits in clinical diagnostic. Each elevated afamin concentration level by 10 mg/l was related to an increase in the prevalence of type 2 diabetes mellitus, while afamin protein was also determined to be positively associated with insulin resistance. The data of this study concluded that the afamin levels are positively and significantly related to insulin resistance and type 2 diabetes

mellitus therefore afamin can be used as a novel marker for the detection of type 2 diabetes (Kollerits et al., 2017).

According to the last new study that the afamin protein level in T1DM lower than the healthy children when compared it, according to this study Adropin, afamin, and neudesin are regulatory peptides may play a large role in the regulation of glucose metabolism especially carbohydrate metabolism, There are a few article papers about afamin in type 1 diabetes mellitus in children (Polkowska et al., 2019)

The aim of the study was to found concentration of afamin protein level and demonstrate it to investigate the suitability of using afamin as an early marker for the detection of T2 DM and T1 DM, Also Compare the results of the afamin protein concentration between type1 and type2 diabetes patients.

## 2. Materials and methods

The study was a case-control study consisted of 220 blood samples aged from (2 – 81) years (Male 115 female 105). During the period from January 2020 to September 2020. Collected samples include 130 patients with diabetes mellitus having the clinical symptoms were diagnosed by laboratory tests such as blood glucose and HbA1c, who were treated at Laila Qasim center for diabetic patients in Erbil City from Kurdistan region, which divided into two equal groups (65) of type 1 DM and (65) of type 2 DM , in addition to intact 90 volunteers without clinical signs of any diseases used as a control, after taking informed consent a standard questionnaire was filled out for the patients and controls, Statistical analysis was based on anthropometric parameters such as age, height, and weight and laboratory tests (HbA1c) using standard methods we included children and adults for both group case and control

Serum was separated to perform by Enzyme-linked immunosorbent assay (ELISA) (ELx800-BioTeK80, U.S.A) in Medical Research Center we used human afamin (AFM) kit produced by fine test Biotech Company to examine the association of afamin protein with diabetes mellitus (company, 2020).

### Statistical Analysis

Statistical Package for Social Science (SPSS) software version 26 was used for data analysis. , while quantitative variables were stated as the

mean standard deviation (SD) . A P value at  $p \leq 0.05$  was measured statistically significant (Di Leo and Sardanelli, 2020).

### 3.Results:

The healthy group was (n=90) and the diabetic group also categorized into two types of patients type2 (n=65) and type1 (n=65) diabetes, which control was two groups, therefore the study populations group divided into four groups which were separated to type 1 diabetes with control of type 1 and type 2 diabetes with control of type 2.

Table 1 shows that the mean age of type 1 participants ( $13.88 \pm 4.34$ ) years and controls of type 1 were ( $14.95 \pm 9.9$ ) years, about mean age T2DM were ( $52.82 \pm 11.92$ ) years and controls of type 2 were ( $43.44 \pm 15.71$ ) years.

The study results showed the different afamin concentration level ranges when compare type 1 and type 2 diabetes with the control group, Table 2. Shows that the significant results of elevated afamin concentration in type 2 diabetes by (9.5 mg/L) compared control group the mean serum afamin in T2DM were ( $75.09 \pm 10.09$  mg/L) and control group was ( $65.62 \pm 7.18$  mg/L). About type 1 diabetes the result showed a significant result that the mean afamin protein levels in type 1 diabetes participants were ( $58.47 \pm 8.13$ ) this value lower by (4.6 mg/L) than the mean of a control group for type 1 diabetes ( $63.07 \pm 5.53$ ).

According to table 3 the biochemical tests which were glucose, glycated hemoglobin (HbA1c), and albumin level in type 1, diabetes and control group of type 1 DM. The results demonstrate statically significant ( $p < 0.001$ ) for both glucose and (HbA1C). The means glucose for patient and control were ( $232.77 \pm 97.33$ ), ( $91.85 \pm 12.1$ ) and the means for HbA1C for both groups were ( $9.18 \pm 2.06$ ), ( $4.93 \pm 0.65$ ) respectively but about albumin level statically was non-significant ( $p > 0.05$ ), the means of albumin level for T1DM and control were ( $4.71 \pm 0.59$ ), ( $4.72 \pm 0.466$ ).

Table 4 showed glucose, glycated hemoglobin (HbA1c), and albumin level in type 2 diabetes with the control group of type 2 the results describe statically significant ( $p < 0.001$ ) for both glucose and (HbA1C). The means glucose for patient and control were ( $253.38 \pm 128.78$ ), ( $99.48 \pm 11.35$ ) with the means for HbA1C for both groups were ( $9.26 \pm 2.12$ ), ( $5.5 \pm 0.39$ ), but about albumin level statically was non-significant ( $p < 0.01$ ), the means of albumin level for T2DM and

control were ( $4.17 \pm 0.455$ ), ( $4.56 \pm 0.65$ ) respectively.

The data of body mass index (BMI) in table 5 for both types of diabetes and controls the results of mean BMI in type 1 diabetes group and control group were ( $19.12 \pm 3.15$ ), ( $18.85 \pm 3.54$ ) respectively. Results statically non-significant ( $p > 0.05$ ), BMI measured significantly higher among T2DM patients than control group data shows statically significant ( $p < 0.001$ ) the means were ( $28.54 \pm 3.7$ ), ( $24.36 \pm 2.4$ ) respectively.

### 4.Discussion

The present study demonstrated that serum afamin concentration has related to diabetes mellitus especially type 1 and type 2 diabetes. And this results is confirmed by many scientific papers.

Afamin concentrations have been founded in various other types of diabetes except type 1 and type 2 diabetes such as gestational diabetes mellitus (Koninger et al., 2018). Afamin protein expression was investigated in various disease studies such as carcinoma which were breast cancer and thyroid cancer (Wang et al., 2020). Relate to this protein more studied done about it which are metabolic syndrome patients and diabetes especially type 2 diabetes but about type 1 diabetes we evaluated afamin level in children, till now have little information about it, Further research is needed about afamin level in type 1 diabetes (Kronenberg et al., 2014).

Data showed that the afamin level was increased in type 2 diabetes patients mean overexpressing human afamin in type 2 diabetes, in the other side the results of our analysis show that the mean afamin concentration were statistically lower children with diabetes type 1 compared to the control group, the results in our study found that the afamin concentration was significantly associated with diabetes mellitus.

Our results were in agreement with another studies results were done about type 2 diabetes before which confirmed that overexpression of serum afamin by (9.5 mg/l) in our results the mean afamin concentration was ( $75.09 \pm 10.09$  mg/l) in type 2 diabetes while in the healthy individual was ( $65.62 \pm 7.18$ ) our data close to another papers data (Kollerits et al., 2017).

Metabolic syndrome another factor that correlated with type 2 diabetes, metabolic syndrome is a combination of type 2 diabetes and cardiovascular

disease (CVD) including dyslipidemia, hyperglycemia and insulin resistance, abdominal obesity, and hypertension these factors have a role in elevated afamin concentration according to this study that the afamin is a promising novel marker for metabolic syndrome and related diseases, therefore by increasing obesity or body mass index (BMI) with type 2 diabetes serum afamin concentration increase, table 5 show that the mean (BMI) in T2DM was  $(28.54 \pm 3.7)$  which is confirm with Kronenberg F. study (Kronenberg and Dieplinger, 2015). About type 1 diabetes mellitus from table 2 data showed that the mean serum afamin was lower than the control group by (4.6 mg/l), the explanation for our results afamin protein act as regulators of glucose metabolism according to a study which supports our data of

afamin in type 1 diabetes a drop-in, afamin, and neudesin may play a major role in the glucose metabolism regulation (Polkowska et al., 2019).

Another criteria we founded from the mean age of table 1 was the comparison between the ages of both types of diabetes, the study described that the type 1 diabetes mellitus age in the early stages of life occur in children while T2DM occurs in adult age after 45 years (Leslie et al., 2016). The means age of type 1 and type 2 diabetes were  $(13.88 \pm 4.34)$  and  $(52.82 \pm 11.924)$  respectively. Because the exact cause unknown there are several causes first of all genetic factor and body's immune system in early age for type 1 DM (Leslie et al., 2016).

**Table 1. The mean age of control and diabetic patients**

Age ( Years)	Control and patient	N	Mean $\pm$ SD	Minimum	Maximum
	Type 1 diabetesx	65	13.88 $\pm$ 4.34	6	25
	Control type1	40	14.95 $\pm$ 9.905	2	41
	Type 2 diabetes	65	52.82 $\pm$ 11.924	20	80
	Control type2	50	43.44 + 15.71	19	81

**Table 2. Serum afamin concentraion in diabetes patient and control participant**

Afamin level Mg/L	Control and patient	N	Mean $\pm$ SD	P-value
	Type 1 diabetes	65	58.47 $\pm$ 8.13	< 0.01
	Control type1	40	63.07 $\pm$ 5.53	
	Type 2 diabetes	65	75.09 $\pm$ 10.09	< 0.001
	Control type2	50	65.62 $\pm$ 7.18	

**Table 3. The levels of serum glucose, albumin, and blood (HbA1C) in type 1 DM and control groups**

	Type1 diabetes N=65 Mean ± SD	Control N=40 Mean ± SD	P-value
Glucose (mg/dl)	232.77 ± 97.33	91.85 ± 12.1	< 0.001
HbA1C (%)	9.18 ± 2.06	4.93 ± 0.65	< 0.001
Albumin (g/dl)	4.71 ± 0.59	4.72 ± 0.466	> 0.05

**Table 4. Levels of serum glucose, albumin, and blood (HbA1C) in type 2 DM and control groups**

	Type2 diabetes N=65 Mean ± SD	Control N=50 Mean ± SD	P-value
Glucose (mg/dl)	253.38 ± 128.78	99.48 ± 11.35	< 0.001
HbA1C (%)	9.26 ± 2.12	5.5 ± 0.39	< 0.001
Albumin (g/dl)	4.17 ± 0.455	4.56 ± 0.65	< 0.01

**Table 5. Comparison of BMI in type 1 and type 2 DM with control groups**

	Control and patient	N	Mean ± SD	P-value
Body Mass Index (BMI) kg/m <sup>2</sup>	type 1 diabetes	65	19.12 ± 3.15	> 0.05
	control type1	40	18.85 ± 3.54	
	type 2 diabetes	65	28.54 ± 3.7	< 0.001
	control type2	50	24.36 ± 2.4	

### 5. Conclusion

This study concluded according to the results that the serum afamin concentration was found significantly higher in type 2 diabetes mellitus group patients than healthy individuals these results help us to use afamin as a biomarker with diabetes mellitus especially with type 2 diabetes

as well as in type 1 diabetes our study demonstrate that the afamin concentration decreased compare to healthy child's, also we need further research to determine afamin level in type 1 diabetes, in the other side, the result showed that the persons with high body mass index, especially in type 2 diabetes mellitus, has more afamin concentration than normal BMI persons.

## 6.Recommendation

We can use afamin as a biomarker with diabetes mellitus especially with type 2 diabetes.

About afamin level in type 1 diabetes has a few studies to confirm our result we need further research to obtain significant data or use as a biomarker for type 1 diabetes patients. The metabolic syndrome is defined as the coexistence of several risk factors for both type 2 diabetes mellitus and cardiovascular disease. Therefore preventative and recommendation need to avoid the causes of metabolic syndrome by aggressive lifestyle modification focusing on weight-loss and physical activity.

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## Reference:

- COMPANY, F. T. 2020. Human AFM(Afamin) ELISA Kit [Online]. Available: <https://www.fn-test.com/product/eh2493/> [Accessed].
- DELHI, N. J. R. T. O. D. M. N. E. N. D. N. D. J. B. M. P. 2012. textbook of diabetes mellitus. 2nd ed, Jaypee Brothers Medical Publishers
- DI LEO, G. & SARDANELLI, F. J. E. R. E. 2020. Statistical significance: p value, 0.05 threshold, and applications to radiomics—reasons for a conservative approach. 4, 1-8.
- JERKOVIC, L., VOEGELE, A. F., CHWATAL, S., KRONENBERG, F., RADCLIFFE, C. M., WORMALD, M. R., LOBENTANZ, E. M., EZEH, B., ELLER, P., DEJORI, N., DIEPLINGER, B., LOTTSPEICH, F., SATTLER, W., UHR, M., MECHTLER, K., DWEK, R. A., RUDD, P. M., BAIER, G. & DIEPLINGER, H. 2005. Afamin is a novel human vitamin E-binding glycoprotein characterization and in vitro expression. *J Proteome Res*, 4, 889-99.
- KOLLERITS, B., LAMINA, C., HUTH, C., MARQUES-VIDAL, P., KIECHL, S., SEPPALA, I., COOPER, J., HUNT, S. C., MEISINGER, C., HERDER, C., KEDENKO, L., WILLEIT, J., THORAND, B., DAHNHARDT, D., STOCKL, D., WILLEIT, K., RODEN, M., RATHMANN, W., PAULWEBER, B., PETERS, A., KAHONEN, M., LEHTIMAKI, T., RAITAKARI, O. T., HUMPHRIES, S. E., VOLLENWEIDER, P., DIEPLINGER, H. & KRONENBERG, F. 2017. Plasma Concentrations of Afamin Are Associated With Prevalent and Incident Type 2 Diabetes: A Pooled Analysis in More Than 20,000 Individuals. *Diabetes Care*, 40, 1386-1393.
- KONINGER, A., MATHAN, A., MACH, P., FRANK, M., SCHMIDT, B., SCHLEUSSNER, E., KIMMIG, R., GELLHAUS, A. & DIEPLINGER, H. 2018. Is Afamin a novel biomarker for gestational diabetes mellitus? A pilot study. *Reprod Biol Endocrinol*, 16, 30.
- KRONENBERG, F. & DIEPLINGER, H. J. C. L. 2015. Afamin is a promising novel marker for metabolic syndrome and related diseases. *Clinical Lipidology*, 10, 207-3.
- KRONENBERG, F., KOLLERITS, B., KIECHL, S., LAMINA, C., KEDENKO, L., MEISINGER, C., WILLEIT, J., HUTH, C., WIETZORREK, G., ALTMANN, M. E., THORAND, B., MELMER, A., DAHNHARDT, D., SANTER, P., RATHMANN, W., PAULWEBER, B., KOENIG, W., PETERS, A., ADHAM, I. M. & DIEPLINGER, H. 2014. Plasma concentrations of afamin are associated with the prevalence and development of metabolic syndrome. *Circ Cardiovasc Genet*, 7, 822-9.
- LESLIE, R. D., PALMER, J., SCHLOOT, N. C. & LERNMARK, A. J. D. 2016. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. 59, 13-20.
- LICHENSTEIN, H. S., LYONS, D. E., WURFEL, M. M., JOHNSON, D. A., MCGINLEY, M. D., LEIDLI, J. C., TROLLINGER, D. B., MAYER, J. P., WRIGHT, S. D. & ZUKOWSKI, M. M. 1994. Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding protein gene family. *J Biol Chem*, 269, 18149-54.
- POLKOWSKA, A., PASIEROWSKA, I. E., PASLAWSKA, M., PAWLUCZUK, E. & BOSSOWSKI, A. 2019. Assessment of Serum Concentrations of Adropin, Afamin, and Neudesin in Children with Type 1 Diabetes. *Biomed Res Int*, 2019, 6128410.
- SEEBER, B., MORANDELL, E., LUNGER, F., WILDT, L. & DIEPLINGER, H. 2014. Afamin serum concentrations are associated with insulin resistance and metabolic syndrome in polycystic ovary syndrome. *Reprod Biol Endocrinol*, 12, 88.
- VOS, T., FLAXMAN, A. D., NAGHAVI, M., LOZANO, R., MICHAUD, C., EZZATI, M., SHIBUYA, K., SALOMON, J. A., ABDALLA, S. & ABOYANS, V. J. T. L. 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. 380, 2163-2196.
- WANG, W. K., TSAI, C. H., LIU, Y. W., LAI, C. C., HUANG, C. C. & SHEEN-CHEN, S. M. 2020. Afamin expression in breast cancer. *Asian J Surg*, 43, 750-754.
- ZHENG, Y., LEY, S. H. & HU, F. B. 2018. Global aetiology and epidemiology of type 2 diabetes

- mellitus and its complications. *Nat Rev Endocrinol*, 14, 88-98.
- COMPANY, F. T. 2020. Human AFM(Afamin) ELISA Kit [Online]. Available: <https://www.fn-test.com/product/eh2493/> [Accessed].
- DELHI, N. J. R. T. O. D. M. N. E. N. D. N. D. J. B. M. P. 2012. textbook of diabetes mellitus. 2nd ed, Jaypee Brothers Medical Publishers
- DI LEO, G. & SARDANELLI, F. J. E. R. E. 2020. Statistical significance: p value, 0.05 threshold, and applications to radiomics—reasons for a conservative approach. 4, 1-8.
- JERKOVIC, L., VOEGELE, A. F., CHWATAL, S., KRONENBERG, F., RADCLIFFE, C. M., WORMALD, M. R., LOBENTANZ, E. M., EZEH, B., ELLER, P., DEJORI, N., DIEPLINGER, B., LOTTSPREICH, F., SATTLER, W., UHR, M., MECHTLER, K., DWEK, R. A., RUDD, P. M., BAIER, G. & DIEPLINGER, H. 2005. Afamin is a novel human vitamin E-binding glycoprotein characterization and in vitro expression. *J Proteome Res*, 4, 889-99.
- KOLLERITS, B., LAMINA, C., HUTH, C., MARQUES-VIDAL, P., KIECHL, S., SEPPALA, I., COOPER, J., HUNT, S. C., MEISINGER, C., HERDER, C., KEDENKO, L., WILLEIT, J., THORAND, B., DAHNHARDT, D., STOCKL, D., WILLEIT, K., RODEN, M., RATHMANN, W., PAULWEBER, B., PETERS, A., KAHONEN, M., LEHTIMAKI, T., RAITAKARI, O. T., HUMPHRIES, S. E., VOLLENWEIDER, P., DIEPLINGER, H. & KRONENBERG, F. 2017. Plasma Concentrations of Afamin Are Associated With Prevalent and Incident Type 2 Diabetes: A Pooled Analysis in More Than 20,000 Individuals. *Diabetes Care*, 40, 1386-1393.
- KONINGER, A., MATHAN, A., MACH, P., FRANK, M., SCHMIDT, B., SCHLEUSSNER, E., KIMMIG, R., GELLHAUS, A. & DIEPLINGER, H. 2018. Is Afamin a novel biomarker for gestational diabetes mellitus? A pilot study. *Reprod Biol Endocrinol*, 16, 30.
- KRONENBERG, F. & DIEPLINGER, H. J. C. L. 2015. Afamin is a promising novel marker for metabolic syndrome and related diseases. *Clinical Lipidology*, 10, 207-3.
- KRONENBERG, F., KOLLERITS, B., KIECHL, S., LAMINA, C., KEDENKO, L., MEISINGER, C., WILLEIT, J., HUTH, C., WIETZORREK, G., ALTMANN, M. E., THORAND, B., MELMER, A., DAHNHARDT, D., SANTER, P., RATHMANN, W., PAULWEBER, B., KOENIG, W., PETERS, A., ADHAM, I. M. & DIEPLINGER, H. 2014. Plasma concentrations of afamin are associated with the prevalence and development of metabolic syndrome. *Circ Cardiovasc Genet*, 7, 822-9.
- LESLIE, R. D., PALMER, J., SCHLOOT, N. C. & LERNMARK, A. J. D. 2016. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. 59, 13-20.
- LICHENSTEIN, H. S., LYONS, D. E., WURFEL, M. M., JOHNSON, D. A., MCGINLEY, M. D., LEIDLI, J. C., TROLLINGER, D. B., MAYER, J. P., WRIGHT, S. D. & ZUKOWSKI, M. M. 1994. Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding protein gene family. *J Biol Chem*, 269, 18149-54.
- POLKOWSKA, A., PASIEROWSKA, I. E., PASLAWSKA, M., PAWLUCZUK, E. & BOSSOWSKI, A. 2019. Assessment of Serum Concentrations of Adropin, Afamin, and Neudesin in Children with Type 1 Diabetes. *Biomed Res Int*, 2019, 6128410.
- SEEBER, B., MORANDELL, E., LUNGER, F., WILDT, L. & DIEPLINGER, H. 2014. Afamin serum concentrations are associated with insulin resistance and metabolic syndrome in polycystic ovary syndrome. *Reprod Biol Endocrinol*, 12, 88.
- VOS, T., FLAXMAN, A. D., NAGHAVI, M., LOZANO, R., MICHAUD, C., EZZATI, M., SHIBUYA, K., SALOMON, J. A., ABDALLA, S. & ABOYANS, V. J. T. L. 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. 380, 2163-2196.
- WANG, W. K., TSAI, C. H., LIU, Y. W., LAI, C. C., HUANG, C. C. & SHEEN-CHEN, S. M. 2020. Afamin expression in breast cancer. *Asian J Surg*, 43, 750-754.
- ZHENG, Y., LEY, S. H. & HU, F. B. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*, 14, 88-98.