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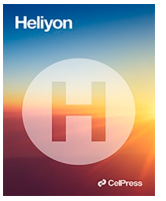

Research article

Circulating afamin positively correlated with the miR-122 expression and type 2 diabetes mellitus-related phenotype according to the duration of diabetes

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ABSTRACT

Background: Afamin is a hepatokine that involves in glucose and lipids metabolism. miR-122 is mainly expressed in liver and involves in lipid and carbohydrate metabolism. This study aimed at investigating the circulating afamin, its correlation with type 2 diabetes mellitus (T2DM) and miR-122 gene expression in T2DM patients and healthy control subjects according to the duration of diabetes.

Methods: This case-control study included 220 participants, with 100 individuals serving as controls and 120 individuals diagnosed with type 2 diabetes mellitus (T2DM). The miR-122 gene expression was assessed using real-time PCR. The serum concentration of biochemical parameters such as glucose levels, lipid profile, and small-dense low-density lipoprotein (sdLDL) were measured using colorimetric kits. Circulating afamin and insulin levels were assayed using an ELISA kit. Glycated hemoglobin (HbA1c) was measured using capillary electrophoresis.

Results: Circulating afamin level was significantly higher in T2DM patients compared to the control group, (73.8 ± 10.8 vs. 65.9 ± 8.7, respectively; $P < 0.001$). Similarly, miR122 expression was significantly increased in T2DM patients compared to healthy control subjects (4.24 ± 2.01 vs. 1.00 ± 0.85, respectively; $P < 0.001$). Among patients diagnosed with T2DM, those with longstanding diabetes (>5 years) exhibited significantly higher levels of circulating afamin and miR-122 expression compared to individuals with a shorter duration of diabetes (≤5 years) ($P < 0.05$). Circulating afamin levels were significantly correlated with waist circumference, small-dense low-density lipoprotein (sdLDL), fasting blood sugar (FBS), insulin, resistance to insulin, and miR-122 expression, depending on the duration of the disease ($P < 0.05$). Furthermore, the performance of afamin as a diagnostic marker for T2DM was confirmed through receiver operating characteristic (ROC) analysis, yielding an area under the curve (AUC) of 0.7 ($P < 0.001$).

Conclusions: Circulating afamin involved in the T2DM-related complications and its concentration is positively correlated to the miR-122 expression, especially in patient with longstanding diabetes.

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1. Introduction

Afamin, also known as vitamin E binding protein, is a glycoprotein that was discovered in 1994 and characterized as the fourth member of the albumin gene family [1]. Human afamin gene is located on chromosome 4q13-3 and most abundantly is expressed in the liver and released into the blood circulation [2]. The physiological and pathophysiological roles of afamin are not yet clearly defined. However, radioligand assay described multiple vitamin E-binding site at human afamin protein and highlighted its role as a carrier of vitamin E [3]. Aafamin plays a crucial role in various biological processes, including fertility, the transportation of vitamin E through the blood-brain barrier, bone metabolism and turnover, and the protection of neuronal cells from harm [4–7]. Significantly, previous population-based study has been demonstrated that afamin may be used as a biomarker for metabolic disorders [8]. A study involving over 20.000 subjects suggested that afamin concentration not only has a considerably association with insulin resistance but also has a diagnostic potential for the type 2 diabetes mellitus (T2DM) [9]. Furthermore, increased afamin concentration was found in gestational diabetes mellitus (GDM) [10–12] and pre-eclampsia patients [13,14]. The concentration of Afamin remains stable regardless of age, gender, fasting state, or circadian cycle. This stability makes it a valuable biomarker in patients with T2DM [15]. Undoubtedly, factors modulating afamin gene expression are as important as afamin itself and can affect our understanding of T2DM pathophysiology and its therapeutic approaches.

MicroRNAs (miRNAs) are a main class of non-coding RNA (ncRNA) involved in the regulation of gene expression at the post-transcriptional stages. miRNA is able to target the 3′ untranslated region (3′UTR) of mRNAs and thereby suppress their translation [16,17]. miR-122 abundantly expressed in liver [18,19] and involved in the regulation of glucose and lipid metabolism [20,21]. Previous studies on the mice model and nonhuman primates showed that miR-122 silencing promotes fatty acid oxidation, decreases lipid synthesis and finally reduces total cholesterol levels [21–23]. Further studies on humans revealed that miR-122 dysregulation may be associated with metabolic disorders [21]. Accordingly, Sengupta D et al. demonstrated that miR-122 involves in homeostasis of liver metabolism and also differentiation of hepatocyte. Their findings also confirmed that inhibition of miR-122 resulted in development of nonalcoholic steatohepatitis [24]. In addition, Willeit P et al. described that circulating miR-122 is correlated with the development of metabolic syndrome and T2DM [25]. A significant correlation between circulating afamin and miR-122 has been previously described. This study aimed at investigating the impact of circulating afamin and miR-122 on the T2DM-related phenotype as well as their correlation based on the duration of diabetes.

2. Materials and methods

2.1. Study population

In this study, 120 patients diagnosed with T2DM (mean age, 56.8 ± 9.06 years) were included. Additionally, 100 non-diabetic individuals were selected as a control group, matched for age and sex (mean age, 55.3 ± 7.6 years). Type 2 diabetes mellitus was diagnosed based on the criteria set by the American Diabetes Association [26].

Diabetic patients were divided into patients with diabetes duration ≤5-years (Group-A) and those with diabetes duration >5-years (Group-B). Patients with gestational diabetes mellitus (GDM), chronic liver and kidney disorders, malignancy, thyroid diseases, and those taking insulin, were excluded. The control group consisted of volunteers who visited health centers for routine laboratory tests. Inclusion criteria for control group included non-pregnancy, FBS<100 mg/dl, HbA1c<5.7%, age≥35 and no history of diabetes in first-degree relatives. All individuals involved provided written informed consent, indicating their voluntary agreement. Moreover, Research Ethics Committee of Abadan University of Medical Sciences approved the study (code of ethics: IR. ABADANUMS. REC.1397.002).

2.2. Sample collection and measurement of biochemical parameters

Peripheral blood samples were collected from each subject after a 12-h fasting period. Biochemical parameters in serum sample including FBS, LDL-C, triglyceride (TG), total cholesterol and high density lipoprotein cholesterol (HDL-C) were measured by a Hitach-912 Autoanalyser (Roche, Switzerland) with colorimetric kits (Pars Azmoon, Tehran, Iran). Concentration of sdLDL was measured using a precipitation method [27]. Briefly, the serum sample (0.1 ml) was mixed with the precipitation reagent (150 U/ml of heparin sodium salt and 90 mmol/l MgCl2) and then incubated for 10 min at 37 °C. After that, the samples were cooled in an ice bath for 5 min and were centrifuged at 15,000 rpm, 4 °C for 15 min. Finally, the supernatant contained sdLDL was measured using LDL-C assay kit (Pars Azmoon, Tehran, Iran). Glycated hemoglobin (HbA1c) and insulin concentration were assayed using capillary electrophoresis and an ELISA kit (Monobind Inc, USA) respectively.

2.3. Measurement of afamin concentration

Serum afamin concentration was measured using ELISA kit (cat. number: RD194428100R, BioVendor, Asheville, NC, USA), according to the recommendations of the manufacturer.

2.4. RNA extraction and cDNA synthesis

miRNA was extracted from whole blood samples using miRNeasy kit (Thermo Fisher scientific, USA, Cat# K157001) based on the manufacturer's instructions. RNA spike-in (synthetic *Caenorhabditis elegans* miRNAss cel-miR-39) was used as an exogenous miRNA to overcome the sample to sample variation during extraction process. Then, the cDNA was synthesized using cDNA synthesis kit (Takara, Biotechnology, Japan).

2.5. Quantitative real-time PCR

Circulating miR-122 was quantified with SYBR Premix Ex *Taq*II(TaKaRa Biotechnology, Japan) using an Exicycler™ 96 (Bioneer, Daejeon, Korea). Quantitative real-time reactions were performed in 25 μl final volume containing 12.5 μl SYBR Green Master Mix, 2 μl cDNA, 1 μl of each primer and 8.5 μl DEPC water. U6 miRNA was used as a reference gene. The primer sequences were as follows; miR-122, sense: 5′-TATCGCCATGATATACGACACAAAC-3′, anti-sense: 5′-GCCGTGGGAGTGGACCATGGT-3′, U6, sense: 5′-CGCTTGCGCGACACATATAC-3′, and anti-sense: 5′-AAATATGACACTCTCACGA-3'. PCR cycling conditions were as follow: 95 °C for 30 s, progressed by 40 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min. Relative gene expression was measured using $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical analysis

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago-USA). Graph Pad Prism version 6.0 (Graph Pad Software, San Diego-USA) was used to preparation of graph. Data was tested for normality using a Kolmogorov-Smirnov test. The statistical differences of quantitative parameters between the two groups were considered by student t-test. The association between variables was evaluated using Pearson's correlation coefficient. Afamin performance as a diagnostic marker was evaluated by Receiver operating characteristic (ROC) analysis. Sensitivity and specificity were calculate based on the Youden's index. Statistical differences were significant when $P < 0.05$.

3. Results

3.1. Demographic and biochemical characteristics of the study population

Table 1 shows the baseline characteristics of the study population. There were no significantly differences in distribution of age, sex, body mass index (BMI), waist circumference, hip circumference and waist-to-hip ratio (WHR) between patients with T2DM and control subjects ($P > 0.5$). T2DM patients showed higher triglycerides (TG) and sdLDL, and lower HDL-C compared with healthy subjects ($P < 0.05$). Additionally, FBS, HbA1c, insulin, and HOMA levels were significantly higher in T2DM patients than those in control group ($P < 0.01$). Table 2 shows the demographic and biochemical characteristics of T2DM patients according to the duration of diabetes. There were no significant differences in terms of age and sex among the subgroups of patients ($P > 0.05$). Regarding anthropometric parameters, Group B shown significantly higher values for waist circumference and waist-to-hip ratio compared to Group A ($P < 0.05$). Lipid profile analysis revealed that mean concentrations of LDL-C and sdLDL in Group-B were significantly higher in comparison to Group-A ($P < 0.05$). Furthermore, Group-B had higher level of FBS and insulin ($P < 0.01$).

Table 1
Biochemical and demographic characteristics of the study population.

Variables	Controls (n = 100)	T2DM patients (n = 120)	*P*-value
Age (year)	55.3 ± 7.6	56.8 ± 9.06	0.1
Sex (male/female)	52/48	65/55	0.7
BMI (kg/m^2)	25.18 ± 6.1	26.5 ± 5.3	0.08
Waist circumference (cm)	92.07 ± 13.5	94.9 ± 15.4	0.1
Hip circumference (cm)	99.9 ± 16.1	104.2 ± 18.4	0.07
Waist-hip-ratio	0.93 ± 0.16	0.93 ± 0.2	0.29
LDL-C (mg/dl)	92.7 ± 20.3	97.05 ± 18.1	0.06
sdLDL (mg/dl)	12.1 ± 2.6	14.7 ± 4.5	**0.00**
HDL-C (mg/dl)	45.8 ± 8.7	42.3 ± 9.4	**0.00**
TG (mg/dl)	144.3 ± 22.4	156.8 ± 25.7	**0.00**
Total cholesterol (mg/dl)	171.7 ± 15.9	175.4 ± 20.4	0.1
Fasting blood sugar (mg/dl)	84.5 ± 10.39	165.5 ± 24.8	**0.00**
HbA1c (%)	4.8 ± 1.2	9.1 ± 1.5	**0.00**
Insulin (μ IU/ml)	6.04 ± 1.2	10.9 ± 2.06	**0.00**
HOMA-IR	1.2 ± 0.31	4.4 ± 1.00	**0.00**

Data are presented as mean ± SD. BMI; Body Mass Index, sdLDL; small dense low-density lipoprotein, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; Triglycerides, HOMA; homeostasis model assessment of insulin resistance, HbA1c; glycated hemoglobin.

Table 2
Biochemical and demographic characteristics of patients with T2DM.

Variables	Time since T2DM diagnosis (year)		*P*-value
	≤5 (Group -A, n = 62)	>5 (Group –B, n = 58)	
Age (year)	57.3 ± 9.2	56.3 ± 8.8	0.5
Sex (male/female)	34/28	31/27	0.87
BMI (kg/m^2)	25.8 ± 4.8	27.2 ± 5.7	0.13
Waist circumference (cm)	90.1 ± 14.3	100.06 ± 15.1	**0.00**
Hip circumference (cm)	106.09 ± 17.8	102.1 ± 19.1	0.2
Waist-hip-ratio	0.86 ± 0.17	1.00 ± 0.2	**0.00**
LDL-C (mg/dl)	93.1 ± 17.2	101.1 ± 18.3	**0.01**
sdLDL (mg/dl)	13.3 ± 3.4	16.4 ± 5.05	**0.00**
HDL-C (mg/dl)	41.5 ± 10.4	43.2 ± 8.1	0.3
TG (mg/dl)	156.02 ± 21.9	157.6 ± 29.4	0.7
Total cholesterol (mg/dl)	178.3 ± 21.4	172.3 ± 19.1	0.07
Fasting blood sugar (mg/dl)	159.19 ± 19.6	172.27 ± 28.1	**0.00**
HbA1c (%)	9.1 ± 1.6	9.1 ± 1.3	0.37
Insulin (μ IU/ml)	10.3 ± 1.9	11.6 ± 2.00	**0.00**
HOMA-IR	4.34 ± 0.94	4.5 ± 1.05	0.18
Afamin (μg/ml)	68.4 ± 9.2	79.6 ± 9.4	**0.00**
miR-122 fold change	3.8 ± 1.7	4.6 ± 2.1	**0.04**

Data are presented as mean ± SD. BMI; Body Mass Index, sdLDL; small dense low-density lipoprotein, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; Triglycerides, HOMA; homeostasis model assessment of insulin resistance, HbA1c; glycated hemoglobin.

3.2. Afamin concentration in study population

Fig. 1, illustrated the results of afamin serum concentration in study population. Afamin concentration in T2DM patients was significantly higher than that in control subjects (73.8 ± 10.8 vs 65.9 ± 8.7) ($P < 0.001$). Sub-group analysis in T2DM patients revealed that Group-B had significantly higher circulating afamin than the Group-A ($P < 0.01$) (Table 2). In diabetic patients, the correlation between afamin, anthropometric and biochemical parameters was evaluated, considering the metabolic role of afamin (Table 3). Among the anthropometric parameters, a positive and significant correlation was observed between circulating afamin and waist circumference in both Group A and Group B ($r = 0.33$, $P < 0.01$ and $r = 0.27$, $P < 0.05$, respectively). However, the correlation remained significant only in Group A after adjustment of BMI, sex, and age ($r = 0.29$, $P < 0.05$). In Group-B, LDL-C was positively correlated with the afamin concentration ($r = 0.31$, $P < 0.001$), but this correlation was not significant after adjustment. Furthermore, group-A showed a positive correlation between circulating afamin and sdLDL, before ($r = 0.36$, $P < 0.01$) and after ($r = 0.32$, $P < 0.05$) adjustment for BMI, sex and age. In Group B, regardless of age, BMI, and sex, there were positive and significant correlations between afamin concentration and various parameters, including FBS ($r = 0.55$, $P < 0.01$), insulin ($r = 0.4$, $P < 0.01$), HOMA-IR ($r = 0.44$, $P < 0.01$), and miR-122 fold change ($r = 0.5$, $P < 0.01$).

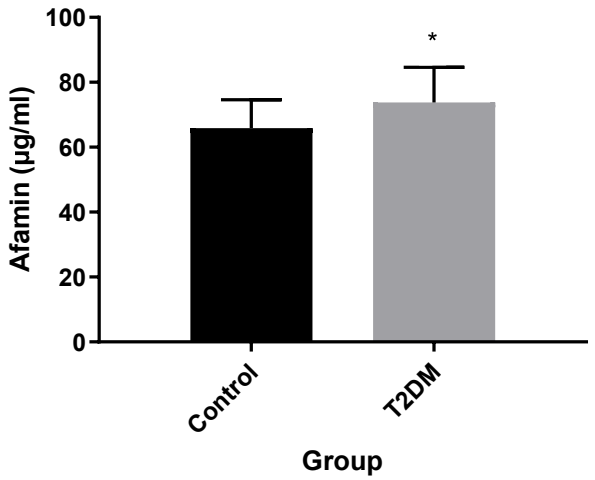

Fig. 1. The afamin concentration in serum of patients with type 2 diabetes mellitus (T2DM) in comparison to healthy control subjects. Data are presented as mean ± SD. *$P < 0.001$ compared to control group.

Table 3
Correlation between circulating afamin, the anthropometric and biochemical parameters in T2DM patients according to duration of diabetes.

Variables	Time since T2DM diagnosis (year)				Time since T2DM diagnosis (year)			
	≤5 (Group –A)		>5 (Group-B)		≤5 (Group-A)		>5 (Group-B)	
	r	*P*-value	r	*P*-value	r[a]	*P*-value[a]	r[a]	*P*-value[a]
Waist circumference (cm)	**0.33**	**0.00**	**0.27**	**0.03**	**0.29**	**0.02**	0.26	0.05
Hip circumference (cm)	0.18	0.14	0.006	0.96	0.19	0.15	0.03	0.79
Waist-hip-ratio	0.09	0.45	0.19	0.15	0.04	0.73	0.24	0.07
BMI (kg/m^2)	0.16	0.2	0.19	0.14	–	–	–	–
LDL-C (mg/dl)	0.05	0.69	**0.31**	**0.01**	0.006	0.92	0.25	0.05
sdLDL (mg/dl)	**0.36**	**0.00**	0.21	0.1	**0.32**	**0.01**	0.18	0.1
Total cholesterol (mg/dl)	0.02	0.75	0.06	0.6	0.01	0.88	0.05	0.7
HDL (mg/dl)	0.01	0.92	0.12	0.34	0.05	0.67	0.16	0.23
TG (mg/dl)	0.04	0.87	0.11	0.4	0.02	0.86	0.04	0.7
FBS (mg/dl)	0.14	0.25	**0.55**	**0.00**	0.13	0.31	**0.52**	**0.00**
Insulin (μ IU/ml)	0.2	0.11	**0.4**	**0.00**	0.19	0.13	**0.4**	**0.00**
HOMA-IR	0.1	0.43	**0.44**	**0.00**	0.07	0.56	**0.42**	**0.00**
HbA1c (%)	0.12	0.18	0.01	0.8	0.18	0.12	0.02	0.8
miR-122 fold change	0.03	0.7	**0.5**	**0.00**	0.03	0.8	**0.54**	**0.00**

BMI; Body Mass Index, sdLDL; small dense low-density lipoprotein, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; Triglycerides, HOMA; homeostasis model assessment of insulin resistance, HbA1c; glycated hemoglobin. [a] Values calculated after adjustment for BMI, age and sex.

3.3. *miR-122 gene expression and its association with afamin concentration*

The result of gene expression analysis is shown in Fig. 2. We found that miR-122 is significantly over-expressed in T2DM patients compared to healthy control subjects (4.24 ± 2.01 vs 1 ± 0.85) ($P < 0.001$). On the contrary, the analysis of the data, considering the duration of diabetes, demonstrated a significant increase in miR-122 expression among individuals with longstanding diabetes compared to those with a shorter duration (4.6 ± 2.1 vs 3.8 ± 1.7, $P < 0.05$). Furthermore, the analysis revealed a positive and significant correlation between miR-122 gene expression and circulating afamin in patients with T2DM ($r = 0.45$; $P < 0.001$) (Fig. 3).

3.4. *Evaluation the diagnostic potential of afamin according to the ROC analysis*

The diagnostic potential of afamin for T2DM was evaluated using ROC analysis (Fig. 4). The area under the curve (AUC) was 0.7 ($P < 0.001$). For a cut-off value 66.05 μg/ml, specificity and sensitivity were 56% and 75%, respectively (95% CI, 0.63–0.77).

4. Discussion

The current study had two main objectives: first, to investigate the levels of circulating afamin and its association with the

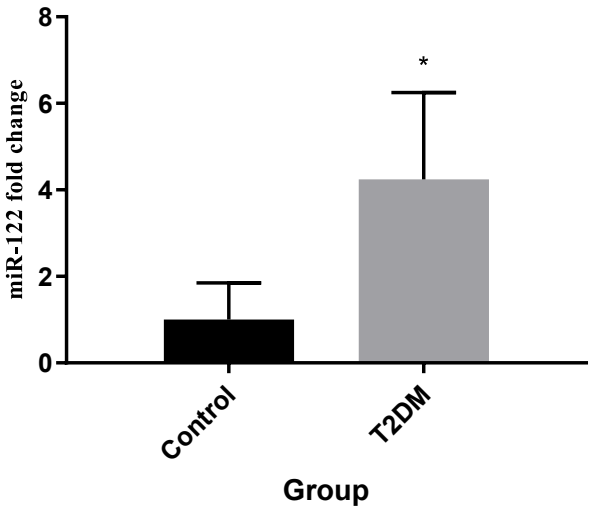

Fig. 2. Comparison of miR-122 fold change between patients with type 2 diabetes mellitus (T2DM) and healthy control subjects. Data are presented as mean ± SD. *$P < 0.001$ in comparison to control group.

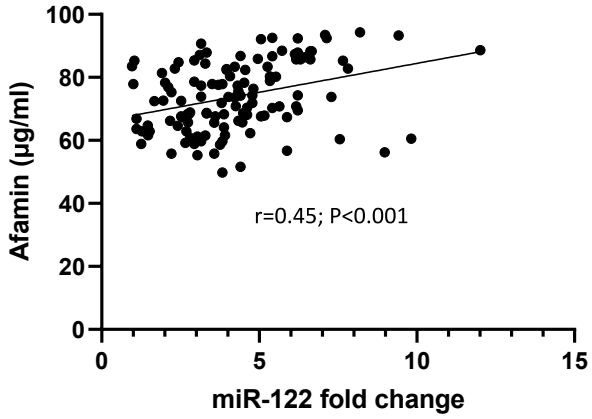

Fig. 3. Correlation between afamin concentration and miR-122 gene expression in patients with type 2 diabetes mellitus (T2DM).

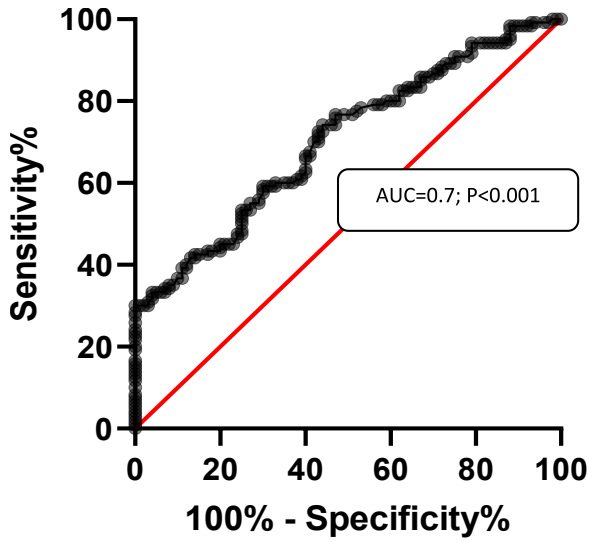

Fig. 4. The ROC curve analysis for the diagnosis of type 2 diabetes mellitus (T2DM) according to the afamin concentration. The area under the curve (AUC) was 0.7. The specificity and sensitivity were 56% and 75% respectively. The cut-off point was afamin >66.05 µg/ml.

biochemical parameters in patients with T2DM and matched control subjects and secondly, to investigate the association between miR-122 gene expression with the afamin concentration according to duration of diabetes. The results revealed a noteworthy rise in afamin concentration among patients diagnosed with T2DM. Additionally, we observed a correlation between afamin levels and dyslipidemia, hyperglycemia, as well as various anthropometric characteristics associated with T2DM. Especially, this study provided evidence that miR-122 over-expression in T2DM patients positively correlated with the circulating afamin. However, ROC analysis confirmed that afamin has a diagnosis potential for T2DM. Remarkably, we found that circulating afamin concentration, miR-122 fold change and their association with T2DM-related complications is dependent to the duration of diabetes.

Afamin is a glycoprotein with ability to bind vitamin E in body fluid [11,28]. Conversely, vitamin E is a powerful antioxidant and its role in dealing with oxidative stress and apoptosis in pancreatic cells has been confirmed [10,29]. Therefore, it can be concluded that afamin is contributed to the oxidative stress condition [30,31]. Given the association between oxidative stress and T2DM, it is expected that there would be an increase in the level of afamin in individuals diagnosed with T2DM [29]. In study by Ali et al., afamin concentration in patients with T2DM and T1DM was compared to the healthy control subjects [1]. Their study highlighted the significantly increased circulating afamin in T2DM patients compared to the controls. Another population-based study conducted by Kollerits et al. involving over 20,000 subjects showed that afamin concentration considerably increased in T2DM patients [9]. In addition to T2DM, Koninger et al. evaluate the serum concentration of afamin in women with GDM during the first trimester [10]. Their findings showed a notable elevation in serum afamin concentration among women who experienced Gestational Diabetes Mellitus (GDM), in comparison to women without GDM. In our study, we made a novel discovery by establishing a correlation between the level of circulating afamin and the duration of diabetes. Specifically, we found that individuals with a longer duration of diabetes exhibited higher concentrations of afamin compared to those with a shorter duration of the disease.

According to the role of afamin in the metabolism of lipids and glucose, its correlation with T2DM-related complication is also important. We observed that not only there is a considerably correlation between afamin levels and T2DM-related complications, but also this correlation is intensified with increasing duration of diabetes. In study conducted by Kurdiova et al. the serum afamin concertation and its metabolic role in different stages of metabolic disorders were investigated [32]. Their results showed that not only afamin concentration significantly increased in individual with prediabetes and T2DM, but also it had a positive correlation with the several T2DM-related phenotypes. Kronenberg et al. investigated the association between afamin with the metabolic syndrome criteria in transgenic mice involving 5000 subjects in epidemiological studies [8]. Their study showed a positive and significant association between serum concentration of afamin with the metabolic syndrome-associated parameters including; values of waist circumference, BMI, systolic and diastolic pressure, LDL-C, total cholesterol and glucose. The association between afamin concentration with T2DM-related phenotypes also described in previous population-based study [9]. Chen et al. also reported that in patients with non-alcoholic fatty liver disease (NAFLD) the serum concentration of afamin is positively correlated with the BMI, waist circumference and also TG levels [33]. The mechanism of association between afamin levels and T2DM-related phenotypes remains to be elucidated. However, this association is debatable from several aspects. Afamin as a hepatokine is mostly expressed in liver under scenario which regulated by nuclear factors 1a and 1b [34]. Decreased circulating afamin in patients with severe alcoholic liver cirrhosis and with liver cancer, highlighted the association between liver function and afamin concentration [35–37]. It has been described that correlation between afamin and T2DM-related complication is contributing to the its role in hepatic lipid accumulation and also inducing resistance to insulin in liver [32]. Direct involvement of afamin in metabolism of glucose in thyroid carcinoma cell line has been described by Shen et al. [38]. Their study indicated that afamin could involve in glucose metabolism through the regulating the expressions key enzymes including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glucose transporter 1(GLUT1) and Hexokinase-2 (HK2). Wnt/c-Jun *N*-terminal kinase signaling pathway has a crucial role in resistance to insulin, inflammatory response, and also dysfunction of pancreatic β-cells [39]. It has also been described that afamin is able to bind to Wnt and lock it in its active form and thereby promotes its role in resistance to insulin and β-cells dysfunction as two indicators of T2DM and metabolic syndrome [40, 41]. The association between increased circulating afamin and T2DM-induced dyslipidemia relies on two principles. First, increased afamin concentration is paralleled by lipid accumulation in liver and fatty liver index [32] and second, afamin is able to acts as a transfer protein and thereby is contribute to exchanging lipoproteins, such as cholesterol, triglycerides, and apolipoprotein B [5]. In spite of all these hypothesizes, there is still a question about the detailed mechanism of association between afamine and the diabetes -related phenotypes.

Our findings described that afamin has a potential to be a diagnostic marker for T2DM. Based on our results, Bendary et al. detected increased serum afamin as the important predictor of postprandial hyperglycemia at 24th gestational week and also development of GDM in women without pregnant diabetes [42]. Kaburagi et al., investigated urinary proteins associated with the diabetic nephropathy using proteomic analysis. Their results showed that AUC value of afamin/creatinine ratio considerably higher than that of albumin/creatinine ratio and described that afamin/creatinine ratio is a suitable predictor of diabetic nephropathy progression [43]. In another study by Chen et al., the diagnostic performance of circulating afamin for NAFLD was evaluated. ROC analysis in their study also confirmed that afamin had an appropriate diagnostic value for NAFLD [33]. The pattern of change in circulating afamin in diabetic patients according to the duration of the disease has not been investigated to yet. Our study provided this idea that circulating afamin probably increases with the duration of diabetes.

miR-122 over-expression in T2DM patients compared to the control subjects was demonstrated in the present study. In T2DM patients, miR-122 expression was dependent on the duration of diabetes. The correlation between miR-122 gene expression with the circulating afamin in T2DM patients was investigated in our study. Accordingly, in patients with longstanding diabetes, miR-122 positively correlated with the afamin concentration.

In line with our findings, dysregulation of miR-122 in T2DM patients was confirmed by previous studies. In study conducted by Willeit el al., increased circulating miR-122 and its positive correlation with afamin was found in patients with metabolic syndrome as well as T2DM [25]. Our previous study also confirmed up-regulation of miR-122 in T2DM patients compared to healthy control subjects [44]. miRNA profiling in T2DM patients by microRNA Array screening also showed miR-122 up-regulation in diabetic patients and also described its role in development of T2DM [45]. This study represents the first documented evidence of the up-regulation of miR-122 and its correlation with circulating afamin, specifically in relation to the duration of diabetes. Accordingly, increasing the duration of the disease was accompanied by an increase in miR-122 expression. However, the mechanism that linked circulating afamin to miR-122 expression requires further investigation.

The main limitation of this study is the relatively small sample size.

5. Conclusion

In conclusion, the findings suggest that circulating afamin plays a role in T2DM-related complications and is associated with miR-122 expression, particularly in patients with longstanding diabetes. Therefore, targeting the afamin/miR-122 may hold therapeutic potential as an approach for managing diabetes. Additionally, afamin shows therapeutic potential diagnostic for diabetes.

Funding statement

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Data availability statement

The datasets analyzed during the present study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Abnoos Mokhtari Ardekani: Writing – original draft. **Ebrahim Kharazinejad:** Data curation. **Ehsan Ghasemi:** Data curation. **Hassan Ghasemi:** Supervision. **Rahmatollah Soltani:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestsHassan ghasemi reports financial support was provided by Abadan University of Medical Sciences. Hassan Ghasemi reports a relationship with Abadan University of Medical Sciences that includes: employment. Hassan Ghasemi has patent pending to Hassan Ghasemi. There are no other relationship or activity that may be interpreted as a conflict of interest by the reader If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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