

Unmasking silent atherosclerosis in type 2 diabetes: the emerging role of adipocyte fatty acid-binding protein

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Objectives. Atherosclerosis contributes to higher mortality and morbidity in people with diabetes. Preventing future complications largely depends on the early detection of imminent atherosclerosis. This study aimed to explore the relationship between the subclinical atherosclerosis measured by carotid intima-media thickness (CIMT), ankle-brachial index (ABI), and circulating levels of adipocyte fatty acid-binding protein (AFABP) in individuals with type 2 diabetes mellitus (T2DM).

Methods. A total of 80 participants evenly divided into two groups: 40 newly diagnosed patients with T2DM and 40 healthy controls. Patients with T2DM had no prior history or clinical signs of macrovascular complications, such as coronary artery disease, cerebrovascular stroke or peripheral artery disease. Serum total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were measured. Plasma AFABP levels were determined using a human enzyme-linked immunosorbent assay (ELISA) kit. ABI was assessed using a sphygmomanometer with a cuff that fits the limb's circumference and a portable Doppler device. Doppler ultrasound of the carotid artery was used to measure CIMT.

Results. In patients with T2DM, circulating AFABP levels were significantly higher than those in healthy subjects ($p=0.007$). Multivariate regression analysis indicated that AFABP is the only independent factor influencing the maximum CIMT ($\beta=0.004$, 95% CI: 0.001–0.007, $p=0.017$).

Conclusion. Plasma AFABP level could serve as a biomarker for early diagnosis of atherosclerosis in patients with T2DM.

Keywords: type 2 diabetes mellitus, atherosclerosis, carotid intima-media thickness, adipocyte fatty acid-binding protein

It is projected that 366 million individuals worldwide will develop diabetes mellitus (DM) by 2030 with type 2 diabetes mellitus (T2DM) accounting for the vast majority of these occurrences (GBD 2021 Diabetes Collaborators 2023; World Health Organization 2023; NCD Risk Factor Collaboration 2024). Long-term organ damage, dysfunction, and even

failure – mostly in the kidneys, eyes, nerves, heart, and blood vessels – are associated with persistently high blood glucose levels in diabetic individuals (Zaccardi et al. 2016).

Atherosclerosis, a chronic inflammatory condition, can begin in early childhood and develop into obstructive arterial disease. When atherosclerotic

lesions progress to a clinically significant stage, severe acute consequences such as myocardial infarction, stroke, and ischemic heart disease may occur (Nelson *et al.* 2019).

Adipocyte fatty acid-binding protein (AFABP), which makes up about 6% of all soluble proteins in adult adipocytes, is highly expressed in these cells and plays a crucial role in controlling inflammation and energy metabolism (Furuhashi 2019). Pathological alterations in the coronary arteries can be predicted by AFABP mRNA expression in homozygous individuals (Tuncman *et al.* 2006). These findings suggest that AFABP may play a role in the development of atherosclerotic disorders (Furuhashi 2019).

Serum AFABP levels were found to be closely associated with key indicators of metabolic syndrome (MetS) including insulin resistance, hyperglycemia, dyslipidemia, and persistently high blood pressure. Additionally, a strong correlation between AFABP and obesity has been demonstrated (Xu *et al.* 2006). Furthermore, AFABP plays a significant role in the onset of T2DM and atherosclerosis (Jing *et al.* 2014).

For this reason, we aimed to assess circulating AFABP levels in patients with T2DM compared to healthy controls and determine its relationship with subclinical atherosclerosis.

Materials and Methods

Study design. This case-control study was conducted at the outpatient clinic of the Diabetes and Metabolism unit at Alexandria Main University Hospital, Egypt. The present study involved a total of 80 participants evenly divided into two groups: Group I included 40 newly diagnosed patients with T2DM according to American Diabetes Association (ADA) criteria (ADA Professional Practice Committee 2024), and Group II, consisting of 40 healthy individuals serving as a control group. Cohen's *d* formula was used for sample size calculation using G*power 3.1.9.7 software, alpha error 0.05, power.9, and an effect size of 0.5.

The study included patients with T2DM with no prior history or clinical signs of macrovascular complications, such as coronary artery disease, cerebrovascular stroke or peripheral artery disease. Additionally, patients with severe uncontrolled hypertension or cardiac decompensation were excluded from the study. The study was conducted in accordance with the Declaration of Helsinki (World Medical Association 2013) and approved by the local Ethics Committee of the Faculty of Medicine of Alexandria

University (IRB NO:00012098, serial number: 0106247, date of approval: 16/01/2020). Informed consent was obtained from all individual participants included in the study.

Study protocol. A detailed history was taken and a clinical examination was performed including measurements of blood pressure and anthropometric data. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height (in meters) squared [$BMI = \text{Weight (kg)} / \text{Height}^2 \text{ (m}^2\text{)}$]. To estimate waist circumference (WC), the midpoint between the iliac crest and the lowest edge of the rib cage was used. Hip circumference (HC) was measured at the point where the buttocks were at their widest. The waist-to-hip ratio was determined by dividing the WC by the HC.

Serum total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were measured. Plasma AFABP levels were determined using a human enzyme-linked immunosorbent assay (ELISA) kit.

Blood samples were collected following standard venipuncture protocols. Each participant provided three samples in the morning after an 8-h fast. The first sample was placed into a lavender top vacutainer tube with EDTA to measure HbA1c. The second sample was added to a grey top vacutainer tube with sodium fluoride to assess FPG. The third sample was transferred into a red top vacutainer tube without any chemicals or anticoagulants. The blood was centrifuged after a 15-min coagulation period. The serum was then collected and stored at -80°C until further testing.

We used a sphygmomanometer with a cuff that fits the limb's circumference and a portable Doppler device operating at 8 MHz to assess the ankle-brachial index (ABI). The brachial artery, posterior tibial, and dorsalis pedis arteries were used to measure the highest systolic blood pressure (SBP). The ABI was determined according to previously described methodology (Alvim *et al.* 2018). To perform the Doppler measurement of carotid intima-media thickness (CIMT), the patient was evaluated while lying supine with a pillow under their head with their head extended and inclined approximately thirty degrees. The hypoechoic and hyperechoic layers located closer to the artery lumen form the intima-media complex (IMC). The posterior wall of the common carotid artery showed IMC in B-mode grayscale with 0.1 mm as the minimum CIMT measurement unit. The average CIMT was measured

in the left and right common carotid arteries (Aminbakhsh and Mancini 1999).

Statistical analysis. SPSS 26 for Windows® software was used for statistical data analysis. Numbers and percentages were used to represent qualitative data. The Chi-Square (or Fisher's exact) test compared two groups with qualitative data. When comparing the two groups, the Student's t-test was applied for quantitative variables that were normally distributed. The Mann-Whitney test was used for quantitative variables that were not normally distributed. The Spearman coefficient was used to correlate two dispersed abnormally distributed quantitative variables. Data were presented as mean $\pm$ standard deviation (SD) and range. Pearson's or Spearman's

correlation was used to analyze the relationship between numeric variables. P values less than 0.05 were considered to be statistically significant.

Results

Cases and healthy control subjects were matched for age and sex. In patients with T2DM, the prevalence of smoking was significantly higher than in controls ($p=0.008$). WC, waist-to-hip ratio, and SBP were all significantly higher in Group I ($p=0.006$, $p=0.009$, and $p=0.001$, respectively) (Table 1).

Regarding the laboratory investigations, AFABP ($p=0.007$), FPG ($p<0.001$), HbA1C ($p<0.001$), serum TG level ($p<0.001$), and LDL-C levels ($p=0.034$)

Table 1
Comparison of the demographic, clinical data, and laboratory parameters between group I and II

Variables	Group I (N=40)	Group II (N=40)	Statistical test	p
Sex				
Male [N (%)]	19 (47.5)	14 (35.0)	$\chi^2=1.289$	0.256
Female [N (%)]	21 (52.5)	26 (65.0)		
Age (years)	57.45 $\pm$ 9.03	52.83 $\pm$ 14.08	$t=1.749$	0.085
Smoking				
Non-smokers [N (%)]	22 (55.0)	33 (82.5)	$\chi^2=7.040^*$	0.008*
Smokers [N (%)]	18 (45.0)	7(17.5)		
BMI (kg/m ²)	35.82 $\pm$ 7.27	33.08 $\pm$ 6.66	$t=1.757$	0.083
WC (cm)	104.45 $\pm$ 14.71	93.70 $\pm$ 18.93	$t=2.836^*$	0.006*
Waist-to-hip ratio	1.01 $\pm$ 0.22	0.90 $\pm$ 0.13	$t=2.664^*$	0.009*
SBP (mmHg)	126.25 $\pm$ 12.34	116.25 $\pm$ 13.34	$t=3.481^*$	0.001*
DBP (mmHg)	79.00 $\pm$ 7.78	75.25 $\pm$ 9.60	$t=1.919$	0.059
FPG (mg/dL)	121.5 (111.0–137.5)	96.50 (89.5–104.0)	$U=151.0$	<0.001*
HbA1c (%)	7.67 $\pm$ 0.44	5.40 $\pm$ 0.32	$t=26.373^*$	<0.001*
HbA1c (mmol/mol)	60.3 $\pm$ 2.2	35.5 $\pm$ 1.6		
TG (mg/dL)	168.20 $\pm$ 58.63	109.20 $\pm$ 44.46	$t=5.071^*$	<0.001*
TC (mg/dL)	172.10 $\pm$ 39.45	181.30 $\pm$ 34.18	$t=1.112$	0.270
LDL-C (mg/dL)	105.20 $\pm$ 38.33	122.60 $\pm$ 33.49	$t=2.159^*$	0.034*
HDL-C (mg/dL)	33.28 $\pm$ 6.80	36.88 $\pm$ 7.07	$t=2.321^*$	0.023*
AFABP (ng/mL)	51.57 (42.34–60.70)	43.10 (32.19–50.90)	$U=522.0^*$	0.007*
Mean ABI	1.12 $\pm$ 0.08	1.11 $\pm$ 0.09	$U=784.0$	0.615
Maximum CIMT (mm)	1.40 $\pm$ 0.18	0.51 $\pm$ 0.12	$t=26.800^*$	<0.001*

Data were presented as mean $\pm$ standard deviation (SD) and range. U – Mann-Whitney test; t – Student t test; χ^2 – Chi square test; p – p-value for comparing between the diabetic and control groups; *statistically significant at $p\leq 0.05$. Abbreviations: AFABP – adipocyte fatty acid-binding protein; ABI – ankle-brachial index; BMI – body mass index; CIMT – carotid intima-media thickness; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL-C – high-density lipoprotein cholesterol; HbA1c – glycosylated hemoglobin; LDL-C – low-density lipoprotein cholesterol; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; WC – waist circumference.

were significantly higher in patients with diabetes compared to healthy subjects. The mean HDL level was significantly lower in patients with T2DM ($p=0.023$) (Table 1).

The mean maximum CIMT was significantly higher in patients with T2DM ($p<0.001$) compared to the control group, while there was no significant difference in the mean ABI between the two groups (Table 1).

Table 2 shows that BMI, maximum CIMT, HbA1c, serum TG, total cholesterol, and LDL-C are positively correlated with serum AFABP in patients with diabetes.

In the univariate linear regression analysis (Table 3), HbA1c, total cholesterol, TG, and AFABP were significantly positively associated with maximum CIMT in T2DM patients. Variables that were significant in univariate analysis were entered into the multivariate regression model. After adjustment, AFABP remained the only independent factor associated with maximum CIMT ($\beta=0.004$, 95% CI: 0.001–0.007, $p=0.017$) (Table 3).

Discussion

There is growing recognition of the role that inflammation plays in the pathogenesis of cardiovascular disease (CVD). In obesity, adipose tissue inflammation, especially in the visceral fat depots, contributes to systemic inflammation and promotes the development of atherosclerosis. This adipose-vascular cross-talk is mediated in large part by the lipid chaperone AFABP, which is widely generated by macrophages and adipocytes (Lee *et al.* 2021).

In a community-based cohort, a higher baseline AFABP concentration predicted incident CVD over a median follow-up of approximately 10 years (Chow *et al.* 2013). Moreover, coronary plaque burden in patients with coronary heart disease and coronary calcium score in individuals with T2DM were linked to elevated circulating levels of AFABP concentrations (Bagheri *et al.* 2010; Miyoshi *et al.* 2010).

In the current study, the AFABP was significantly higher in patients with T2DM compared to healthy controls.

This aligns with El-Sayed *et al.* (2019) who have found that the level of AFABP was statistically significantly higher in patients with diabetes compared to the control. Moreover, compared to individuals who have diabetes without MetS, they observed a notable increase in blood AFABP levels in patients who have diabetes with MetS.

In our study, we found a statistically significant positive correlation between AFABP and weight, BMI, HC, HbA1c, TGs, total cholesterol, and LDL-C.

In the same context, Pearson's correlation analysis by Xiao *et al.* (2018), who examined the relationship between plasma AFABP levels and other parameters in 133 participants with T2DM, have shown multiple positive correlations between circulating AFABP and BMI, fasting insulin, and HOMA-IR. Additionally, after adjusting for age and sex, plasma AFABP remained positively correlated with BMI, WC, waist-to-hip ratio, fasting insulin, and HOMA-IR (Xiao *et al.* 2018).

These findings are not surprising, as extracellular vesicles from adipose tissue are probably a main source of circulating AFABP. Therefore, AFABP could serve as a marker for adiposity in clinical settings, which is an important aspect of MetS (Xu *et al.* 2006; Mauvais-Jarvis 2015).

Regarding the subclinical atherosclerosis, we observed a significant positive correlation between CIMT and AFABP. Additionally, AFABP level is an independent factor associated with higher CIMT.

Table 2

Correlation between AFABP and different studied parameters in group I

AFABP vs.	r_s	p
Age (years)	-0.180	0.266
BMI (kg/m ²)	0.467	0.002*
Waist Circumference (cm)	0.212	0.190
Waist-to-Hip ratio	-0.156	0.337
SBP (mmHg)	0.069	0.672
DBP (mmHg)	0.078	0.632
Mean ABI	-0.044	0.789
Maximum CIMT (mm)	0.583	<0.001*
FPG (mg/dL)	0.280	0.080
HbA1c (%)	0.804	<0.001*
TG (mg/dL)	0.395	0.012*
TC (mg/dL)	0.430	0.006*
LDL-C (mg/dL)	0.332	0.036*
HDL-C (mg/dL)	-0.183	0.259

r_s – Spearman coefficient; *Statistically significant at $p \leq 0.05$. Abbreviations: AFABP – adipocyte fatty acid-binding protein; ABI – ankle-brachial index; BMI – body mass index; CIMT – carotid intima-media thickness; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL-C – high-density lipoprotein cholesterol; HbA1c – glycosylated hemoglobin; LDL-C – low-density lipoprotein cholesterol; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; WC – waist circumference.

Table 3
Univariate and multivariate linear regression analysis for the parameters affecting maximum CIMT in group I

Variables	Univariate		*Multivariate	
	p	B (LL–UL 95%CI)	p	B (LL–UL 95%CI)
Age (years)	0.237	0.004 (–0.003–0.010)		
Female	0.960	–0.003 (–0.117–0.112)		
BMI (kg/m ²)	0.497	0.003 (–0.005–0.011)		
Waist-to-Hip ratio	0.067	–0.235 (–0.488–0.018)		
SBP (mmHg)	0.343	0.002 (–0.002–0.007)		
DBP (mmHg)	0.218	0.005 (–0.003–0.012)		
Mean ABI	0.436	–0.272 (–0.971–0.427)		
FPG	0.652	0.0002 (–0.001–0.001)		
HbA1c (%)	0.003*	0.185 (0.068–0.302)		
TC	0.016*	0.002 (0.0003–0.003)	0.428	0.001 (–0.001–0.002)
TG	0.016*	0.001 (0.0002–0.002)	0.828	0.067 (–0.556–0.689)
LDL-C	0.100	0.001 (–0.0002–0.003)		
HDL-C	0.727	0.001 (–0.007–0.010)		
AFABP	0.001*	0.005 (0.002–0.008)	0.017*	0.004 (0.001–0.007)

B – Unstandardized Coefficients; CI – Confidence interval; LL – lower limit; UL – upper limit; #All variables with p. Abbreviations: AFABP – adipocyte fatty acid-binding protein; ABI – ankle-brachial index; BMI – body mass index; CIMT – carotid intima-media thickness; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL-C – high-density lipoprotein cholesterol; HbA1c – glycosylated hemoglobin; LDL-C – low-density lipoprotein cholesterol; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides.

In agreement with our findings, Xiao et al. (2018) have discovered that a high baseline AFABP level was an independent predictor for the development of subclinical atherosclerosis assessed by CIMT in their prospective study, which was conducted on newly diagnosed patients with T2DM followed for 8 years. Additionally, Allam and Tantawi (2021) have reported a significant association between AFABP and CIMT.

The cross-sectional nature of the current trial limited the prediction of future atherosclerotic cardiovascular events and this aspect should be addressed in further studies. AFABP is a key mediator of adipose-vascular communication through both direct and indirect effects, according to recent epidemiological studies. However, from a clinical perspective, more validation research is unquestionably needed to explore the potential of using AFABP as a promising marker for CVD in clinical practice. Additionally, standardizing commercial AFABP ELISA assays is

equally important. While preclinical research has clearly shown that AFABP is a promising therapeutic target for combating CVD, intervention studies to evaluate the safety and effectiveness of pharmacological inhibitors of AFABP are eagerly awaited. Overall, recent research has definitively proven that AFABP is an important adipokine linking obesity with inflammation and poor cardiovascular outcomes, even though its clinical use as a diagnostic or therapeutic target may still be a long way off.

Conclusion

Serum AFABP levels are higher in patients with T2DM and significantly correlate with CIMT. Therefore, AFABP levels could be used as a marker of subclinical atherosclerosis in patients with T2DM.

Conflict of interest: The authors declare no conflicts of interest.

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