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Relationship Between Serum Adipocyte Fatty Acid Binding Protein and Atherosclerosis in Type 2 Diabetes Patients

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ABSTRACT

Background: Atherosclerosis as a complication of diabetes, is considered to be a chronic inflammatory disease, developing over years and proceeding through many steps. Adiposity fatty acid–binding protein (A-FABP) is highly expressed in mature adipocytes and plays a central regulatory role in energy metabolism and inflammation. A-FABP mRNA expression in adipose tissues predicted coronary artery disease in homozygous subjects. A-FABP may also play a role in the development of atherosclerotic diseases in humans.

Objective: To detect the relationship between serum A-FABP level and carotid intima-media thickness (IMT) as indicator of atherosclerosis in patients with type 2 diabetes mellitus.

Patients and methods: 124 patients with type 2 diabetes (T2D), were divided into two groups: Group 1: 62 patients with T2D ≤ 5 years duration, and Group 2: 62 patients with T2D > 5 years duration. They were subjected to full history taking, clinical examination, and laboratory investigations in the form of CBC, LFT, KFT, HBA1c, fasting and postprandial blood sugar, A-FABP serum level, and carotid Doppler.

Results: Serum level A-FABP and carotid IMT was significantly higher in long standing diabetes group compared to short standing diabetes group. A-FABP cutoff was proven to have a high sensitivity 94.4%, specificity 100.0%, positive predictive value of 100.0%, negative predictive value of 97.7% and overall accuracy of 98.3% regarding the prediction of atherosclerosis**.**

Conclusions: A-FABP serum level have a high sensitivity, specificity**,** predictive value and overall accuracy regarding atherosclerosis, and should be recommended as a part of routine surveillance for atherosclerosis in patients with type 2 diabetes.

Keywords: Adipocyte fatty acid protein, Carotid Doppler, Diabetes.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by dis-regulation of carbohydrate, lipid and protein metabolism, and results from impaired insulin secretion, insulin resistance or a combination of both with an increased risk of microvascular and macrovascular disease; its main clinical characteristic is hyperglycemia[(1)]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organ especially the eyes, kidney**,** nerves, heart and blood vessels[(2)].

Atherosclerosis is considered to be a chronic inflammatory disease, begins early in life and over time can eventually lead to obstructive arterial disease. Once atherosclerotic lesions become clinically significant, serious acute complications such as ischemic heart disease, MI and stroke may occur[(3)].

Adipocytes fatty acid–binding protein (A-FABP, also known as aP2 or FABP4) is highly expressed in mature adipocytes, accounting for approximately 6% of their total soluble protein[(4)]. It belongs to the super-family of small molecular weight intracellular lipid-binding proteins, and plays a central regulatory role in energy metabolism and inflammation[(5)]. Although A-FABP was traditionally thought to be an intracellular protein, a small portion of A-FABP is released from mature adipocytes into the human blood stream with the serum concentrations being ranging from (8 to 16 ng/ml) [(6)]. A-FABP mRNA expression in adipose tissues predicts coronary artery disease in homozygous subjects[(7)]. These findings suggest that A-FABP may also play a role in the development of atherosclerotic diseases in humans[(8)].

Serum A-FAB levels are correlated closely with several key features of the metabolic syndrome; an aggregate of cardio metabolic risk factors associated with accelerated atherosclerosis[(9)], including adverse lipid profiles (increased serum triglyceride and LDL-cholesterol, and decreased HDL-cholesterol), insulin resistance, hyperglycemia, and hypertension[(10)]. A-FABP is closely associated with obesity and metabolic syndrome[(11)]. In prospective studies, A-FABP levels predicted the development of metabolic syndrome and type 2 diabetes[(12)].

Individuals with an A-FABP variant had lower triglycerides and a reduced risk of obesity-induced type 2 diabetes[(7)]. Several studies suggested that A-FABP is closely associated with insulin resistance and plays a central role in the development of metabolic syndrome, type 2 diabetes, and atherosclerosis[(13)].

The aim of the work was to detect the relationship between serum A-FABP level and

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carotid intima-media thickness (IMT) as indicator of atherosclerosis in patients with type 2 diabetes mellitus.

PATIENTS AND METHODS

This case controlled study was conducted in Zagazig University Hospitals at the period from June 2019 till January 2020, on 124 patients, divided in two equal groups:

Group 1: 62 patients (37 males, 25 females) their mean age was 50.61±5.58 years, type 2 diabetic patients for less than 5 years.

Group 2: 62 patients (49 males, 13 females) their mean age was 52.31±6.11 years, type 2 diabetic patients for more than 5 years.

Inclusion criteria: Diabetic type 2 patients on oral hypoglycemia, and ages between 35-70 years.

Exclusion criteria: Type 2 diabetic patients on insulin therapy, malignancies, acute or chronic inflammation, and liver disorders.

All patients were subjected to thorough clinical evaluation with emphasis on:

Full medical and surgical history, and general clinical examination.

Laboratory investigations:

Routine investigation: Fasting and 2-hour postprandial blood glucose level, HbA1c, complete blood count, liver function tests, kidney function test and lipid profile.

Specific investigation:

-A-FABP by enzyme-linked immunosorbent assay (ELISA).

-Carotid Doppler for measurement of carotid IMT (mm): High-resolution B-mode ultrasound (Philips Sonos 5500, 2004) was used to measure the IMT of the common carotid arteries (CCA). Linear array transducers with frequency of 10 MHz were used. Anterolateral approach was used to longitudinally image the right and left CCA, Plaque was defined as a focal protrusion into the lumen with a thickness of at least 50% more than adjacent intima-media complex.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study.

Statistical analysis

Data were collected throughout history, basic clinical examination, laboratory investigations and outcome measures were coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) Differences between frequencies (qualitative variables) and percentages in groups were compared by Chi-square test. Quantitative variables were presented as means± standard deviations (SD), median and range. They were compared by independent t-test if parametric and by Mann-Whitney test if nonparametric. P value was set at <0.05 for significant results and <0.001 for high significant results.

RESULTS

The demographic data of the studied groups, age distribution, gender and smoking showed no significant difference between groups. But there was a significant difference between groups regarding BMI being higher in long standing diabetes group compared to short standing group **(**Table 1).

Table (1): Demographic data between studied groups

		Group I N=62	Group II N=62	Total	P
		N (%)	N (%)	N (%)	
Sex	**Male**	37 (59.7%)	49 (79.03%)	86 (69.35%)	0.019*
	Female	25 (40.3%)	13 (20.97%)	38 (30.65%)	
Smoking	**NO**	38 (61.3%)	47 (75.8%)	85 (68.55%)	0.082
	YES	24 (38.7%)	15 (24.2%)	39 (31.45%)	
Age	Mean±SD	51.61±5.58	53.31±6.11		0.108
BMI	Mean±SD	26.1±1.84	27.8±2.29		<0.001**

*: Significant difference **: Highly significant difference

There was a significant difference between groups regarding Hb A1c and HDL being higher in long standing diabetes (group II). Age, BMI, 2 hours postprandial sugar, HbA1C, LDL, triglycerides, cholesterol, serum creatinine, urea, serum level A-FABP; all were significantly higher in long standing diabetes (group II) but HDL was significantly lower (Table 2).

Table (2): Laboratory parameters of the studied groups.

		Group I N=62 Mean±SD	Group II N=62 Mean±SD	P
FBG (mg/dl)		137.55±18.62	138.66±24.69	0.778
PP2 (mg/dl)		217.26±36.6	233.93±41.33	0.019*
Hb A1C		6.22±0.476	6.41±0.54	0.040*
LDL (mg/dl)		120.03±18.12	125.35±23.26	0.158
HDL (mg/dl)		52.83±5.57	47.16±9.8	<0.001**
TG (mg/dl)		121.29±15.5	126.19±30.74	0.265
Cholesterol (mg/dl)		163.9±27.96	169.67±4.11	0.386
AST (U/L)		23.93±4.7	25.16±4.97	0.159
ALT (U/L)		24.21±4.3	25.74±5.48	0.086
Cr (mg/dl)		1.01±0.1	1.03±0.11	0.292
Urea (mg/dl)		35.06±5.11	34.22±7.46	0.466
A-FABP		0.84±0.05	1.55±0.25	0.009*
	Median	0.6	0.83	

*: Significant difference**: Highly significant difference, FBG: fasting blood glucose, pp2: 2 hours post prandial glucose.

Carotid IMT was significantly higher in long standing diabetes (group II) compared to group I (Table 3).

Table (3): Carotid IMT between groups.

		Group I N (%)	Group II N (%)	Total N (%)	P
Doppler Carotid IMT (Atherosclerosis)	**No**	54 (87.1%)	34 (54.8%)	88 (71.0%)	<0.001**
	Yes	8 (12.9%)	28 (45.2%)	36 (29.0%)	
Total		62 (100.0%)	62 (100.0%)	124 (100.0%)	

**: Highly significant difference

The cutoff value, sensitivity, specificity, positive and negative predictive values, and overall accuracy of A-FABP in prediction of atherosclerosis development are shown in tables 4 and 5 and figure 1. In addition there was a significant association and agreement between A-FABP and carotid IMT (Table 5).

Table (4): ROC curve for detection of A-FABP cutoff as regard atherosclerosis

Area under the curve	Cutoff	P	95% Confidence Interval	
			Lower Bound	Upper Bound
0.984	> 1.61	<0.01**	0.951	1.000

**: Highly significant difference

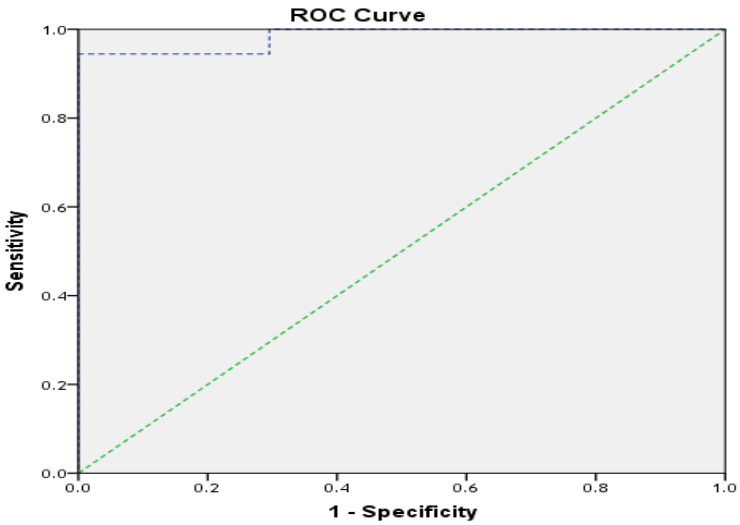

Fig. (1): ROC Curve for detection of A-FABP cutoff as regard atherosclerosis.

Table (5): Association between A-FABP and carotid IMT

		Doppler atherosclerosis		Total N (%)	P	Kappa
		No N (%)	Yes N (%)			
A-FABP	**<1.610**	88 (100%)	2 (5.6%)	90 (72.6%)	<0.001**	0.96
	>1.61	0 (0.0%)	34 (94.4%)	34 (27.4%)		
Total		88 (100%)	36 (100%)	124 (100%)		

Validity

	Sensitivity	Specificity	+ve predictive	-ve predictive	Accuracy
A-FABP Cutoff	94.4%	100.0%	100.0%	97.7%	98.3%

**: Highly significant difference

Family history, smoking and Group II was significantly associated with carotid IMT denoting atherosclerosis (Table 6).

Table (6): Association between carotid IMT and different parameters

		Doppler		Total N (%)	P
		Non N (%)	Atherosclerosis N (%)		
Sex	**Male**	64 (72.7%)	24 (66.7%)	88 (71.0%)	0.450
	Female	24 (27.3%)	12 (33.3%)	36 (29.0%)	
Family history	**-ve**	76 (86.4%)	16 (44.4%)	92 (74.2%)	<0.001**
	+ve	12 (13.6%)	20 (55.6%)	32 (25.8%)	
Smoking	**NO**	68 (77.3%)	18 (50.0%)	86 (69.4%)	0.003*
	YES	20 (22.7%)	18 (50.0%)	38 (30.6%)	
Group	**Group I**	54 (61.4%)	8 (22.2%)	62 (50.0%)	<0.001**
	Group II	34 (38.6%)	28 (77.8%)	62 (50.0%)	
Total		88 (100.0%)	36 (100.0%)	124 (100.0%)	

*: Significant difference **: Highly significant difference

Age, BMI, HbA_1C, and family history were significant independent predictors for atherosclerosis (Table 7).

Table (7): Logistic regression for independent predictors

	P	R	95% C.I.	
			Lower	Upper
Age (year)	0.008*	24.087	2.322	58.64
BMI (kg/m^2**)**	0.001**	32.73	5.897	67.548
HA1C (mg/dL)	0.001**	45.621	12.325	87.654
LDL (mg/dL)	0.055	8.654	0.98	28.654
HDL (mg/dL)	0.052	14.503	0.93	35.654
Triglycerides (mg/dL)	0.097	11.609	0.87	39.254
Cholesterol (mg/dL)	0.087	1.467	0.91	19.54
Family history	0.012*	5.655	1.467	21.805
Smoking	0.134	2.740	0.732	10.255
Group II	0.039*	3.794	1.953	15.107

*: Significant difference **: Highly significant difference

DISCUSSION

Chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction and failure of different organs especially the eyes, kidneys, nerves, heart and blood vessels[2]. Adipocyte fatty acid- binding protein (A- FABP), one of the most interesting adipocytokines secreted from adipocytes, plays an important role in metabolic deterioration and the development of atherosclerosis[14].

This case controlled study was conducted in Zagazig University Hospitals at the period from June 2019 till January 2020, on 124 patients with type 2 diabetes.

Our results showed non-significant difference between the two groups as regard age, sex and duration of diabetes**,** this is supported by **Alberti *et al.*[15]**.

The BMI was significantly higher in group 2 than group 1 with a significant correlation with the duration of diabetes, which agrees with **Tanamas *et al.*[16].**

Our study showed no association between smoking and duration of diabetes, which is against **Ohkuma *et al.*[17]** who reported that smoking is significantly associated with diabetes duration. This can be interpreted to the fact of our smaller sample size (124 compared to 2490)**.**

In the present study duration of diabetes significantly correlated with Hb A_1C and HDL, same results were reported by **Ohkuma *et al.*[17]**. Also A-FABP values significantly correlated with the duration of diabetes, which is concurrent with **Shore *et al.*[18]**.

Also our results showed that atherosclerosis development (proven by carotid artery Doppler) significantly correlated with the duration of diabetes, which is in agreement with **Parrinello *et al.*[19]**.

After applying proper statistical methods, we concluded that there was significant correlation between serum A-FABP levels and carotid IMT, in agreement with **Karpisek *et al.*[20]**, but against **Jiri *et al.*[21]** who denied any beneficial role of A-FABP as a marker for atherosclerosis.

The A-FABP showed a sensitivity of 94.4%, specificity of 100.0%, +ve predictive value of 100.0%, -ve predictive value of 97.7% and an overall accuracy of 98.3%, in prediction of atherosclerosis development, which is agreed with **Xiao *et al.*[22]**.

Carotid IMT was found to be significantly associated with elevated serum levels of glucose in the 2 hours postprandial measures, Hb A_1C, LDL, HDL triglyceride (TG) and cholesterol, which is agreed with **Stubbs *et al.*[23].** Carotid IMT was found not to be associated with serum levels of aspartate aminotransferase (AST) or alanine aminotransferase (ALT), which is against **Pais *et al.*[24]** findings that might be attributed to the difference in racial background in the study groups.

We found that carotid IMT was significantly associated with smoking and family history, which is in agreement with **Pham *et al.*[25].** Also carotid IMT was significantly associated with the duration of diabetes in agreement with **Reis *et al.* [26].** Another findings is that carotid IMT was not associated with the sex differentiation, which is against **LeBlanc *et al.* [27]** that might be contributed to the fact that we only included postmenopausal women after the loss of estrogen protection.

After applying the logistic regression analyses, age, BMI, Hb A_1C, family history and duration of diabetes were significant independent predictors for atherosclerosis, which correlates with **Yeboah *et al.* [28].**

CONCLUSION AND RECOMMENDATIONS

A-FABP serum level have a high sensitivity, specificity**,** predictive value and overall accuracy regarding atherosclerosis, and should be recommended as a part of routine surveillance for atherosclerosis in patients with type 2 diabetes.

REFERENCES

1. **Zaccardi F, Webb D, Yates T *et al.* (2016):** Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. Postgrad Med J., 92(1084):63-9.
2. **American Diabetes Association (2015):** Diagnosis and classification of diabetes mellitus. Diabetes Care, 38(1): 8-16.
3. **Wang C, Hess C, Hiatt W *et al.* (2016):** Atherosclerotic cardiovascular disease and heart failure in type 2 diabetes – Mechanisms, management, and clinical considerations. Circulation, 133(24): 2459–2502.
4. **Makowski L, Brittingham K, Reynolds J *et al.* (2005):** The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and Ikappa B kinase activities. J Biol Chem., 280:12888–12895.
5. **Boord J, Fazio S, Linton M (2002):** Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. Curr Opin Lipidol., 13:141-7.
6. **Xu A, Wang Y, Xu J *et al.* (2006):** Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem., 52(3):405–13.
7. **Tuncman G, Erbay E, Hom X *et al.* (2006):** A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. Proc Natl Acad Sci USA., 103:6970–75.
8. **Yeung D, Xu A, Cheung C *et al.* (2007):** Serum adipocyte fatty acid-binding protein levels were

independently associated with carotid atherosclerosis. Arterioscler Thromb Vasc Biol., 27:1796–1802.

9. **Hsu B, Chen Y, Lee R *et al.* (2010):** Fasting serum level of fatty –acid-binding protein 4 positively correlates with metabolic syndrome in patients with coronary artery disease. Circ J., 74:327-31.
10. **Xu A, Tso A, Cheung B *et al.* (2007):** Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation, 115(12):1537–43.
11. **Stejskal D, Karpisek M (2006):** Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? Eur J Clin Invest., 36:621-5.
12. **Tso A, Xu A, Sham P *et al.* (2007):** Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care, 30(10):2667–72.
13. **Jing F, Mao Y, Guo J *et al.* (2014):** The value of Apolipoprotein B/Apolipoprotein A1 ratio for metabolic syndrome diagnosis in a Chinese population: a cross-sectional study. Lipids in Health and Disease, 13: 81-7.
14. **Flink L, Mochari- Greenberger H, Mosca L (2013):** Gender differences in clinical outcomes among diabetic patients hospitalized for cardiovascular disease. Am Heart J., 165: 972– 978.
15. **Alberti K, Zimmet P, Shaw J (2006):** Metabolic syndrome a new world-wide definition. A consensus statement from the International Diabetes Federation. Diabetic Medicine, 23(5): 469–480.
16. **Tanamas S, Wong E, Backholer K *et al.* (2016):** Age of onset of obesity and risk of type 2 diabetes. Australian and New Zealand Journal of Public Health, 40(6): 579-581.
17. **Ohkuma T, Iwase M, Fujii H *et al.* (2015):** Dose-and time-dependent association of smoking and its cessation with glycemic control and insulin resistance in male patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. PLoS One, 10(3): 122023.
18. **Shore A, Helen M, Natali A *et al.* (2018):** Use of vascular assessments and novel biomarkers to predict cardiovascular events in type 2 diabetes: The SUMMIT VIP Study. Diabetes Care, 41(10): 2212-2219.
19. **Parrinello C, Ina R, Godino J *et al.* (2015)**: Prevalence of and racial disparities in risk factor control in older adults with diabetes: the Atherosclerosis Risk in Communities Study. https://care.diabetesjournals.org/content/early/2015/03/31/dc15-0016
20. **Karpisek M, Stejskal D, Hlozankova M *et al.* (2017):** Adipocyte fatty acid-binding protein: A predictive marker of metabolic syndrome. Atherosclerosis, 263: 202-06.
21. **Jiri O, David K, Milan H *et al.* (2019):** Association of serum adipocyte fatty acid-binding protein and apolipoprotein B /apolipoprotein A1 ratio with intima media thickness of common carotid artery in dyslipidemic patients. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 163(2):166-171.
22. **Xiao Y, Xiao X, Xu A *et al.* (2018)** Circulating adipocyte fatty acid-binding protein levels predict the development of subclinical atherosclerosis in type 2 diabetes. Journal of Diabetes and its Complications, 32(12): 1100-1104.
23. **Stubbs J, House J, Ocque A *et al.* (2016):** Serum trimethylamine-N-oxide is elevated in CKD and correlates with coronary atherosclerosis burden. Journal of the American Society of Nephrology, 27(1): 305-313.
24. **Pais R, Giral P, Ratziu V (2017):** Relationship between fatty liver, coronary calcium score, multiple-sites atherosclerosis and 10-years Framingham Score. Journal of Hepatology, 66(1): 588-589.
25. **Pham T, Fujiyoshi A, Hisamatsu T *et al.* (2018):** P2508 Smoking associates with higher incidence and progression of coronary atherosclerosis in a community-based sample of Japanese men: a cohort study. European Heart Journal, 25: 477-489.
26. **Reis J, Allen N, Bancks M *et al.* (2018):** Duration of diabetes and prediabetes during adulthood and subclinical atherosclerosis and cardiac dysfunction in middle age: The CARDIA Study. Diabetes Care, 41(4):731-738.
27. **LeBlanc S, Coulombe F, Bibeau K *et al.* (2018):** Sex-related differences in atherosclerosis burden and composition by magnetic resonance: a complex interaction between visceral adiposity and atherogenic lipoproteins. Canadian Journal of Cardiology, 34(10): 163-164.
28. **Yeboah J, Young R, McClelland R *et al.* (2016):** Utility of nontraditional risk markers in atherosclerotic cardiovascular disease risk assessment. Journal of the American College of Cardiology, 67(2): 139-147.