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Original Article

The association between fetuin-A and testosterone levels and markers of arterial stiffness in Saudi subjects with type 2 diabetes mellitus

Tarek Mohamed Ali[a,b,*], Ahmad El Askary[a,c]

[a] Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Saudi Arabia
[b] Department of Medical Physiology, Faculty of Medicine, Beni-Suef University, Egypt
[c] Department of Medical Biochemistry, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt

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ABSTRACT

Background: Inconsistent results have been described regarding the part of fetuin-A and testosterone in arterial stiffness in type 2 diabetes mellitus (T2DM).
Aim: To look into the links of serum fetuin-A and testosterone levels with brachial-Ankle pulse wave velocity (baPWV), a marker of arteriosclerosis and common carotid intima media thickness (ccIMT), a marker of early atherosclerosis, in diabetic Saudi men patients.
Subjects and methods: One hundred and fifty adult male patients with T2DM and 60 non-diabetic control subjects were enrolled from different Saudi Arabia Taif hospitals. Biochemical analysis, anthropometric measurements, blood pressure, baPWV and ccIMT were investigated.
Results: Stepwise regression in diabetic patients revealed that the most important predictor of ba-PWV was serum fetuin-A followed by serum glucose and the most important predictor of ccIMT was serum fetuin-A followed by serum HDL then serum triglycerides.
Conclusions: Only fetuin-A levels not testosterone are negatively associated with early markers of atherosclerosis.

1. Introduction

Diabetes mellitus (DM) is a chronic illness that is characterized by incapability of the body to produce sufficient or effectively utilize insulin, and is stimulated by a genetic predisposition concurrently with environmental factors [1]. Universally, about 422 million adults existed to live with diabetes in the year 2014, matched to one hundred and eight million in the year 1980. The expected number of people with DM globally is growing and by 2030 this will be augmented to 552 million The predictable number of people with diabetes in the middle east and north Africa (MENA) region is expected to increase to 72% by 2045 [2]. Type 2 diabetes mellitus (T2DM) is accompanied by a higher cardiovascular morbidity and mortality, and risk of by cardiovascular diseases (CVD) compared to non-diabetic subjects [1]. Diabetic vascular disease is cause of the rise in the incidence of CVD [3]. Two categories of vascular modifications are detected, atherosclerotic and arteriosclerotic changes. Atherosclerosis is linked to rise of the arterial intima-media thickness (IMT), leading ultimately to luminal obstruction with subsequent ischemic events, for example, myocardial infarction and stroke. Arteriosclerosis causes arterial stiffening and amplified pulse wave velocity (PWV) and pulse pressure; consequentially this may be the reason of left ventricular hypertrophy and decreased coronary perfusion. In diabetic patients both conditions appear early and follow an enhanced course contributing to the disproportionate cardiovascular mortality in these patient inhabitants [4]. Fetuin-A is a hepatic glycoprotein with multi-functions and utilizes its effects on the cardiovascular system by two different mechanisms. One of these mechanisms is the inhibition of insulin signaling and induction of insulin resistance contributing to the start of atherosclerosis [5]. The other mechanism is the inhibition of calcium deposition and protection from vascular calcification by impeding vascular smooth muscle cell (VSMC) apoptosis and prevention of extracellular matrix basic calcium particle nucleation [6]. As it is known, insulin resistance is a chief pathophysiological mechanism concerned with the development T2DM. Previous documents showed that persons with T2DM have augmented levels of fetuin-A in contrast with individuals without T2DM [7]. Inconsistent results have been described regarding the part of fetuin-A in peripheral arterial disease (PAD) in diabetics. Some of previous studies have stated that fetuin-A levels are lesser in individuals

* Corresponding author at: College of Applied Medical Sciences, Conferences Road, Taif, Saudi Arabia.
E-mail address: tarek70ali@gmail.com (T.M. Ali).

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with T2DM and PAD, while another study described differing findings [8,9].

Dhindsa et al. [10] described that equal to one-third of patients with T2DM would have low serum testosterone levels together with clinical verification of hypogonadism. Furthermore, a higher incidence of hyperinsulinism and reduced insulin sensitivity which are well-known risk factors for the progress of T2DM and CVD [11] either diabetic or nondiabetic persons with decreased serum testosterone. In a previous study, Fukui et al. [12] revealed an inverse relationship between serum endogenous androgen levels and PWV as an indicator of arterial stiffness in men with T2DM. Nevertheless, little is recognized about the above association in patients with T2DM, so we intended to look into the links of serum fetuin-A and testosterone levels with brachial to ankle PWV (baPWV), a marker of arteriosclerosis [13], and common carotid IMT (ccIMT), a marker of early atherosclerosis [14], in a group of diabetic Saudi men patients.

2. Subjects and methods

2.1. Patients

One hundred and fifty adult male patients (age from 49 to 79 yrs.) with T2DM and 60 non diabetic subjects served as a control group (age from 44 to 75 yrs.) were included in the study. All participants were from the different general hospitals in Taif, Saudi Arabia who agreed to the protocol and entered the study consecutively. Inclusion criteria were male gender, age less than 80 years, and no history of cardiovascular disease. Patients with history of cardiovascular events, arrhythmia, cardiomyopathy, valvular heart disease, endocrine sicknesses that could affect the hypothalamic-pituitary-gonadal axis and preceding androgen replacement therapy were excluded from continuing the study. In addition, patients with hepatic diseases were disqualified from the study. The protocol was approved by the Ethical Committee Office of the Scientific Deanship of Taif University (Taif, Saudi Arabia) number 1-2722-434 which was in accordance to guidelines of Helsinki Declaration. All patients signed an informed consent preceding their participation in the current study.

2.2. Laboratory methods

About 5 ml of blood were drawn from a peripheral vein under fasting conditions in the morning between 08:30 and 09:30 a.m. Venous blood samples were assembled from each subject and was left to clot in a serum collecting tube and then centrifuged at $4000 \times g$ for 5 min and serum was separated. Fasting blood glucose (FBG) was determined immediately using the glucose oxidase method on Mindray BS-300 Chemistry Autoanalyzer. The rest of serum was stored at $-20\,^{\circ}C$ for estimation of urea, creatinine, cholesterol, triglycerides, testosterone and fetuin-A. The determination of serum cholesterol, serum triglyceride, and serum urea and serum creatinine was carried out utilizing Mindray BS-300 Chemistry Autoanalyzer using colorimetric methods. Determination of HDL-cholesterol was done using precipitation methods, phosphotungstic acid and magnesium ions are used for precipitating all lipoprotein except the HDL fraction, which was left in the supernatant and measured by Mindray BS-300 Chemistry Autoanalyzer. LDL-cholesterol was estimated by the Friedwald formula [15]. For the assessment of serum testosterone in all subjects included in this study we used the competitive enzyme linked immunoassay (ELISA) technique and analytical procedure was performed using the testosterone ELISA kits purchased from abcam (ab178655, USA). The determination of serum fetuin-A was done using quantitative sandwich enzyme linked immunoassay (ELISA) technique, and the kit was supplied from Thermo Scientific (Frederick, MD 21704- Toll Free in USA).

2.3. Anthropometric measurements

Body weight was taken in subjects dressed in light clothing with no shoes. Height was taken whilst the contributor is in the standing position utilizing a calibrated stadiometer. From the height and weight, the body mass index (BMI) was assessed as weight / $(\text{height})^2$ and expressed as (kg/m^2). It was measured by the same well-trained person. After subjects were rested in a sitting position for nearly ten min, blood pressure was taken from the brachial artery as systolic (SBP) and diastolic (DBP) by automated oscillometric maneuvers (Omron HEM712C; Tokyo, Japan). Mean blood pressure (MAP) was calculated as diastolic blood pressure + 1/3 pulse pressure.

2.4. Measurement of pulse wave velocity

Brachial-Ankle pulse wave velocity (baPWV) was determined by the use of a volume plethysmographic apparatus (model BP-203RPE, Colin, Komaki, Japan) with an intra-observer (10.0%) and an inter-observer (8.4%) reproducibility [16]. The baPWV was valued as the mean of both left and right baPWV. After resting for 15 min, participants were studied in the supine position on a different appointment from blood withdrawal. Waveform information were attained from a volume plethysmographic sensor in cuffs on both brachiums and both ankles, then time intervals (T) between the wave forward-facing the brachiums and that of the ankles were calculated. Rendering to the subject's height, the distance (L) between the heart and selection points was reflected automatically. baPWV = L/T (L = La- Lb) whereas La = the heart - ankle distance, then Lb = the heart - brachium distance [16].

2.5. Measurement of common carotid IMT (ccIMT)

The (cc IMT) was recognized by means of high-resolution B-mode ultrasonography (EnVisor; MA, USA) with a 5–12 MHz transducer and via a specific software for IMT measurement (Intima scope; Media Cross Co.,Tokyo, Japan) at three levels of both carotid artery lateral and medial walls, one-three cm proximal to the branching of common carotid artery. The (cc IMT) was considered as the mean of both left and right IMT values. All measurements were verified by a well-trained professional who was not alert of the participant's anthropometric or laboratory records [17].

2.6. Statistical analysis

Continuous variables are identified as mean ± standard deviation. Each variable was tested for normality of distribution via the Kolmogorov-Smirnov test. Directed at comparisons between control and diabetic groups, study variables were analyzed using independent Student's t-test. All analyses were piloted using Statistical Package for Social Science (SPSS) software ver. 20 (Armonk, NY: IBM Corp.). Pearson's correlation coefficient test was used to define the relationship between plasma fetuin-A, ba-PWV and cc IMT and other study variables in subjects with T2DM. This was depending on whether the variables were normally distributed. Statistical significance was agreed at $p < 0.05$. Step-wise multiple regression analysis was performed to detect the most predictable variables that determine both baPWV and cc IMT in diabetic persons.

Table 1
The clinical and biochemical characteristics of the study groups.

Parameters	Groups				
	Control group		Diabetic group		
	Mean	SD	Mean	SD	P-value
Age (years)	46.30	4.58	46.78	5.74	.088
BMI(kg/m^2)	24.88	1.82	25.04	1.18	.074
SBP (mmHg)	132.78	3.20	133.69	5.17	.174
DBP (mmHg)	84.71	4.00	87.78	4.15	.000
Testosterone (ng/dl)	7.00	2.47	3.42	1.26	.000
Fetuin-A (pg/dl)	71.07	23.48	275.90	58.81	.000
Fasting blood glucose (mg/dl)	72.93	10.14	279.34	91.10	.000
Fasting serum insulin(IU/ml)	5.61	1.57	4.92	1.48	.005
HOMA-IR	2.04	0.36	3.23	1.04	.000
Total Cholesterol (mg/dl)	155.67	19.78	215.69	35.63	.000
HDL (mg/dl)	44.07	1.86	38.25	4.26	.000
LDL-C (mg/dl)	103.60	11.06	222.85	74.68	.000
TG (mg/dl)	100.87	9.19	132.17	35.54	.000
ba-PWV (m/sec)	16.15	1.35	18.95	3.12	.000
ccIMT (mm)	0.72	0.19	0.95	0.21	.000

Values are conveyed as mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; ba-PWV, brachial-ankle pulse wave velocity; ccIMT, Common carotid intima media thickness.

3. Results

3.1. The clinical and biochemical features of the study participants

The clinical and biochemical features of study subjects are displayed in Table 1. Age, BMI and SBP were not significantly different ($p > 0.05$) between control and diabetic groups. DBP, fetuin-A, fasting blood glucose, fasting serum insulin, total cholesterol, HOMA-IR, LDL-C, TG, ba-PWV, and ccIMT were significantly higher in diabetic than in control group ($p < 0.05$). Although HDL and testosterone were significantly lower in diabetic group when compared to control group ($p < 0.05$).

3.2. Correlations between fetuin-A and clinical and biochemical parameters in control and diabetic groups

In control group, fetuin-A level demonstrated a significantly positive relation with serum testosterone ($p < 0.05$) and total cholesterol ($p < 0.01$) while it showed a significant negative relationship with HDL. Although, in diabetic group, it demonstrated a significantly positive association with serum testosterone and HOMA-IR ($p < 0.05$) and SBP, FBG, total cholesterol ($p < 0.01$), LDL-C and TG. Whereas, it was significantly negatively correlated with age, fasting serum insulin, HDL, ba-PWV, and ccIMT ($p < 0.01$) Table 2.

3.3. Correlations between ba-PWV and clinical and biochemical parameters in control and diabetic groups

In control group, ba-PWV revealed a significantly negative relationship with BMI ($p < 0.01$). Even though, in diabetic group, ba-PWV showed a significantly positive association with age, FBG, total cholesterol ($p < 0.01$), LDL-C, TG and ccIMT ($p < 0.01$). However, it revealed a significant negative relationship with serum fetuin-A level and HDL Table 2.

Table 2
Pearson correlation coefficient of fetuin, baPWV and ccIMT to different variables in the study groups.

	Control			Diabetic		
	Fetuin r	baPWV r	ccIMT r	Fetuin r	baPWV r	ccIMT r
Age (years)	.026	−.210	−.237	**−.326-****	**.303****	**.366****
BMI	.035	**−.353-****	−.253	−.146	.095	**.167***
SBP (mmHg)	.066	−.129	−.040	**.425****	.122	**.278****
DBP (mmHg)	.066	−.129	−.040	−.150	.062	.091
Testosterone (ng/dl)	**.292***	.125	−.107	**.180***	−.154	−.088
Fetuin-A (pg/dl)		.078	−.074		**−.487-****	**−.460-****
Fasting blood glucose (mg/dl)	.087	.023	.056	**.471****	**.327****	**.282****
Fasting serum insulin(IU/ml)	−.126	.010	.205	**−.403-****	−.149	**−.216-****
HOMA-IR	.033	−.148	−.221	**.141***	.091	.050
Total Cholesterol (mg/dl)	**.386****	.017	.063	**.435****	**.261****	**.379****
HDL (mg/dl)	**−.465-****	.027	−.071	**−.565-****	**−.177-***	**−.285-****
LDL-C (mg/dl)	.247	−.002	.100	**.391****	**.299****	**.370****
TG (mg/dl)	−.118	−.082	.047	**.622****	**.334****	**.447****
ba-PWV (m/sec)	.078			**−.387-****		
ccIMT (mm)	−.074	.192		**−.460-****	**.282****	

Values are conveyed as mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; ba-PWV, brachial-ankle pulse wave velocity; ccIMT, Common carotid intima media thickness.

** $p < 0.01$ level.

* $p < 0.05$ level.

Table 3
Stepwise regression to study the most fit model describing the predictors of baPWV in diabetic group.

Models						
	Model 1			Model 2		
	B	t	Sig.	B	t	Sig.
Fetuin	−.387	−5.105	.000[b]	−.299	−3.527	.000[c]
Glucose				.186	2.196	
R^2	.150			.177		
R Square Change	.150			.027		
F	26.065			15.780		
F Change	26.065			4.823		
Sig. F Change	.000			.030		
a Dependent Variable: baPWV						

B = Unstandardized coefficients.

3.4. Correlations between ccIMT and clinical and biochemical parameters in control and diabetic groups

In control group, ccIMT was not significantly correlated with any of the studied parameters ($p > 0.05$). Although, in diabetic group, it was positively correlated with BMI ($p < 0.05$), age, SBP, fasting blood glucose, total cholesterol, LDL-C, and TG ($p < 0.01$). While, it showed a significant negative association with serum fetuin-A, fasting serum insulin and HDL ($p < 0.01$) Table 2.

3.5. Stepwise regression to study the most fit model describing the predictors of PWV in diabetic group

A stepwise multiple regression was conducted to evaluate which of the study parameters would predict baPWV in diabetic patients. At step 1 of the analysis serum fetuin-A entered into the regression equation and it a significant association with baPWV where F (1,148) = 26.065, $p < .001$. Model 1 was responsible for approximately 15% of the variance of the baPWV that could be accounted for by serum fetuin-A (R2 =.150). The multiple correlation coefficient was -.39 indicating that 39% of the variance in baPWV is caused by serum fetuin-A. Other variables were excluded by stepwise analysis and deleted from the equation. While at step 2 of the analysis glucose was entered and was significantly related to baPWV where F (2,147) = 15.780, $p < .001$. ($t = 2.196$, $p > .05$). This model was responsible for 18% approximately of the variance of the baPWV (R2 =.18). The most important predictor in this model was serum fetuin-A (correlation coefficient was −.299) followed by serum glucose (correlation coefficient was .186) Table 3.

3.6. Stepwise regression to study the most fit model describing the predictors of ccIMT in diabetic group

A stepwise multiple regression was conducted to evaluate which of the study parameters would predict ccIMT in diabetic patients. At step 1 of the analysis serum fetuin-A included into the regression equation and was significantly associated with ccIMT where F (1,148) = 39.686, $p < .001$. Model 1 was responsible for approximately 21% of the variance of the ccIMT that could be accounted for by serum fetuin-A (R2 =.211). The multiple correlation coefficient was .46 indicating that 46% of the variance in ccIMT in this model is caused by serum fetuin-A. Other variables were excluded by stepwise analysis and omitted from the equation. While at step 2 of the analysis TG was entered and was significantly related to ccIMT where F (2,147) = 25.044, $p < .001$. ($t = 2.901$, $p < .001$). This model was responsible for 25% approximately of the variance of the ccIMT (R2 =.254). The most important predictor in this model was serum fetuin-A (correlation coefficient was -.372) followed by serum TG (correlation coefficient was .225). Although at step 3 of the analysis HDL was entered and it was significantly related to ccIMT where F (3,146) = 18.401, $p < .001$. ($t = -3.022$, $p < .001$). This model was responsible for 27% approximately of the variance of the ccIMT (R2 =.274). The most important predictor in this model was serum fetuin-A (correlation coefficient was −.274) followed by serum HDL (correlation coefficient was −.194) and the last important predictor was serum TG (correlation coefficient was .166) Table 4.

4. Discussion

Type II diabetes is defined by insufficient insulin secretion and insulin resistance in the target tissues. Insulin mediates its action through phosphorylation of the insulin receptor. Fetuin-A inhibits insulin receptor autophosphorylation [18]. Several influences have been confirmed to be connected with arterial stiffness for instance, liver-produced anti-calcificatory hormone, fetuin-A and sex hormone, testosterone [19]. Fetuin-A has ambivalent effects on the cardiovascular system acting as an atherogenic factor via exacerbation of insulin resistance or by way of calcification inhibition. So, we tried to investigate the effects of fetuin-A and testosterone on the markers of early atherosclerosis in male diabetic patients. The current study presented highly significant elevated fetuin-A and decrease serum testosterone compared to control group. In control and diabetic groups, fetuin-A level was significantly positively correlated with serum testosterone. Kim et al, revealed that the most notable outcome of their study is the contribution of sex hormones in the regulation of fetuins. Precisely, the authors found that fetuin-A was strangely increased by

Table 4
Stepwise regression to study the most fit model describing the predictors of ccIMT in diabetic group.

Models									
	Model 1			Model 2			Model 3		
	B	t	Sig.	B	t	Sig.	B	t	Sig.
Fetuin	−.460	−6.300	.000[b]	−.372	−4.806	.000[c]	−.274	−3.022	.000[d]
TG				.225	2.901		.166	2.026	
HDL							−.194	−2.018	
R^2	.211			.254			.274		
R Square Change	.211			.043			.020		
F	39.686			25.044			18.401		
F Change	39.686			8.413			4.071		
Sig. F Change	.000			.004			.045		
d. Dependent Variable: ccIMT									

B = Unstandardized coefficients.

dehydrotestrone (DHT) [20]. Our results showed significantly higher fasting blood glucose, fasting serum insulin and HOMA-IR in diabetic patients and fetuin-A levels showed significantly positive correlations with FBG and HOMA-IR and a negative correlation with fasting serum insulin in these patients. These associations were in agreement with a previous study by Eleftheriadou I et al, who revealed similar results [19]. Also, A previous study reported by Wallace et al. [21], proved that fetuin-A levels were interrelated with fasting insulin levels and HOMA-IR in obese patients, suggesting a potential link between fetuin-A and insulin resistance. The role of fetuin-A in regulation of insulin resistance is determined also by experimental studies as noted that fetuin-A-knockout mice exhibit better insulin sensitivity [22]. Extra well-studied mechanisms have been proposed that fetuin-A worsens insulin resistance by hindering the insulin receptor tyrosine kinase, decreasing the adiponectin expression, and elevating the level of some inflammatory cytokine [23,24]. Consequently, greater levels of fetuin-A may be linked with the pathogenesis of T2D [25]. Nevertheless, Mori et al. did not achieve a significant link between fetuin-A and insulin resistance in T2DM [26]. A previous study [27] found that the relationship between serum fetuin-A and T2DM was modified by the presence of higher glucose levels. The authors observed a positive association between contributors with elevated plasma glucose levels inside the non-diabetic range, while fetuin-A was not associated with diabetes risk amongst participants with normal glucose levels. Regarding the lipid profile and their relationship with fetuin-A in diabetic group, significantly higher total cholesterol, LDL-C and TG and lower HDL were observed. Significant positive correlation were obtained between fetuin-A and total cholesterol, LDL-C and TG, while it showed a negative correlation with HDL. Many previous studies have confirmed this fact. One of these studies verified that the serum fetuin-A levels are entirely associated with visceral obesity besides dyslipidemia [28]. Ix et al. [29], also, stated that higher level of fetuin-A was related to higher triglycerides and LDL-cholesterol. Kotronen and Yki-Jδrvinen [30] exhibited that fetuin-A levels were negatively correlated with HDL-cholesterol. Khalil and Kuobaili [31] conveyed that raised serum fetuin-A levels in T2DM patients were significantly connected with dyslipidemia, thus representing that fetuin-A may be one of the causal factors to the increased prevalence of coronary heart diseases in T2DM patients. Though, in distinction to our study, a previous study described that fetuin-A presented no significant association with the metabolic factors [9]. In the current study, the significantly positive correlation of fetuin-A level and SBP in diabetic group, was in agreement with many studies including a previous study in obese subjects that showed a correlation between serum fetuin-A and SBP and DBP [32]. Also, Jenkins et al. [33]. found in their younger applicants that plasma fetuin-A was significantly connected to blood pressure. In the current report, we found that ba-PWV was significantly negatively correlated with BMI in control group but this association was not found in diabetic group. Even though, in diabetic subjects, ba-PWV was significantly higher compared to non-diabetic subjects. It showed a significant positive correlation with age, fasting blood glucose, total cholesterol, LDL-C, TG and ccIMT. However, it presented a significant negative association with serum fetuin-A level and HDL. A stepwise multiple regression was conducted to evaluate which of the study parameters would predict PWV in diabetic patients. At step 1 of the analysis about 39% of the variance in PWV was caused by serum fetuin-A, while other variables did not enter into the equation and excluded by stepwise analysis. Although, at step 2 of the analysis where glucose was entered, still fetuin-A is the most important predictor of variance in the PWV (30%) followed by serum glucose (19%). In a previous study [34], in only men, fetuin-A revealed an inverse connection to aPWV before ($r=-0.28$, $p=0.025$) and after adjustment of multivariable. Influentially, we found lower levels of fetuin-A to be associated with a higher aPWV in men. Therefore, we have confidence in that the negative association between fetuin-A and aPWV in men may be revealed by the antifibrotic effect of this hepatokine. Nevertheless, Mori et al. stated a positive relationship between fetuin-A levels with arterial stiffness autonomous of known atherogenic issues [35]. In a previous study conducted on 17 diabetic patients, the correlations of cfPWV with fetuin-A did not grasp a statistical significance, while in 64 non-diabetic patients, the above associations showed a high significance [36]. Fetuin-A facilitated prevention of VSMC calcification possibly will hypothetically weaken hemodynamic values of vascular calcification, for instance, arterial stiffening plus increased PWV [36]. The results of preceding studies which examined the above association are unreliable. Mori et al. [35] established an autonomous positive association between fetuin-A and stiffness of the common carotid artery, in healthy Japanese subjects. while Roos et al. [9] described an inverse relationship with aortic PWV in males, but not in females, with normal renal function. The above discrepant results may, nonetheless partly, reveal differences in approach and patient inhabitants. Though, the autonomous negative relationship of cfPWV with fetuin-A is in harmony with its well familiar anti-calcifying effects [37,6], and its described negative relationship with mortality [38]. In current report, in diabetic group, ccIMT was significantly higher and positively correlated with BMI, age, SBP, fasting blood glucose, total cholesterol, LDL-C, and TG ($p<0.01$), while, it was significantly negatively correlated with serum fetuin-A, fasting serum insulin and HDL. A stepwise multiple regression was conducted to evaluate which of the study parameters would predict CAIMT in diabetic patients. From these variables only fetuin-A, TG and HDL were entered in stepwise multiple regression while other variables were excluded. At step 1 of the analysis, 46% of the variance in ccIMT in this model is caused by serum fetuin-A. While at step 2 of the analysis, the most important predictor in this model was serum fetuin-A (37%) followed by serum TG (22.5%). Although at step 3 of the analysis, the most important predictor in this model was serum fetuin-A (27%) followed by serum HDL (19%) and the last important predictor was serum TG (17%). In 17 diabetic patients of a previous study, the associations of ccIMT with fetuin-A did not grasp a statistical significance ($p=0.144$) whereas in 64 non-diabetic patients this relationship was significant. The authors in this study showed that fetuin-A presented a significant negative association with ccIMT, which however, lost significance after adjustment for age. The associations of fetuin-A with cfPWV and ccIMT in this report were significant individually in the non-diabetic persons even though a tendency in the similar direction was also detected in the diabetics. The authors ascribed that to the lesser number of diabetic participants. Studies considering the above relationship are limited and the outcomes are unreliable [36]. A previous study by Guarneri M et al, in essential hypertensive patients with normal kidney function presented an independent negative relationship of fetuin-A and ccIMT [39]. Though, a previous study in community-living persons without CVD failed to exhibit a analogous association [29], despite the fact that in 90 carotid or femoral atherosclerotic patients and conserved renal function a positive association was detected [40]. Caglar et al. presented in non-diabetic patients with CKD stage 1–5 a negative association of fetuin-A with carotid IMT that, nevertheless, mislaid significance in multivariate analysis [41]. Nevertheless in a report by Pertosa et al. [42] baseline fetuin-A levels were inversely and autonomously related to carotid IMT. Roos et al. enrolled 153 patients with early diabetic nephropathy, fetuin-A levels were lesser in patients with PAD, but no association was observed between fetuin-A levels and ccIMT, an initial indicator of atherosclerosis. Owing to their remark the authors advised that fetuin-A may be related to predominant

and not early atherosclerotic illness, even though the exact mechanisms remain indistinct [9]. Variances in procedure and patient population may once more, at least somewhat, explain the discrepant outcomes. Additionally, in disparity to the well-known anti-calcifying special effects of fetuin-A, its link with initial atherosclerotic vascular modifications looks to merit additional investigation. Totally, the available literatures are unreliable regarding whether fetuin-A aggravates or guards against vascular illnesses. Utmost data advocate that fetuin-A has a biphasic outcome on atherosclerosis; as it was found in the early stages it may act as an atherogenic element by means of initiation of insulin resistance, whereas in the latter levels of atherosclerosis it may act defensively as an inhibitor of vascular calcification [43]. Our finding that fetuin-A levels are negatively associated with early markers of atherosclerosis is in line with the assumption that fetuin-A is elevated in patients with T2DM and that in this case fetuin-A may employ its effect as a calcification inhibitor instead of as an atherogenic influence. Whereas, serum testosterone was not an important predicting variable determining early markers of atherosclerosis.

Conflicts of interest

None.

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