

# Lowering Effect of Valsartan on Fetuin-A in Type 1 Diabetes Mellitus

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**Introduction.** Fetuin-A (A2-HS-glycoprotein) is a protein that plays several functions in human physiology and pathophysiology. The role of fetuin-A in type 1 diabetes mellitus (DM) has been less studied. We investigated the serum levels of fetuin-A in type 1 diabetic patients with microalbuminuria. Furthermore, the blocking effect of renin-angiotensin-aldosterone system on serum levels of fetuin-A was assessed.

**Materials and Methods.** From January 2010 to May 2011, 32 patients with type 1 DM with confirmed microalbuminuria were included in this study in Isfahan, Iran. Serum fetuin-A levels before and 8 weeks after valsartan administration were measured. In addition, serum lipid profile, creatinine, hemoglobin A1c, and urine microalbuminuria were determined.

**Results.** The mean age of participants was  $21.65 \pm 0.38$  years. Before valsartan administration, the mean values of fetuin-A were not significantly different between males and females ( $64.22 \pm 1.77$  ng/mL versus  $61.39 \pm 3.35$  ng/mL, respectively). After valsartan administration, serum levels of fetuin-A and urine albumin-creatinine significantly decreased. A negative correlation was observed between serum fetuin-A level after valsartan administration and serum low-density lipoprotein cholesterol level ( $P = .007$ ,  $r = -0.507$ ).  
**Conclusions.** Administration angiotensin receptor blockers concomitantly decreases fetuin-A levels and urine albumin levels.

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## INTRODUCTION

Various inflammatory, metabolic, and procoagulant molecules have been studied to define the microvascular and macrovascular risk of type 2 diabetes mellitus (DM).<sup>1-3</sup> One of these markers that has been relatively less studied is fetuin-A (A2-HS-glycoprotein). Fetuin-A, a 60 kDa protein synthesized in hepatocytes, is a novel marker of vascular diseases.<sup>4</sup> Various functions of fetuin-A in human physiology and pathophysiology is the outstanding feature of this protein.<sup>5</sup> Indeed, fetuin-A may act as an either aggravating or protective factor in cardiovascular diseases.<sup>5</sup> Considering DM, controversial results have been achieved in patients

with type 2 DM and peripheral vasculopathy.<sup>2, 6</sup> The role of fetuin-A in type 1 DM has been less studied. We studied the serum levels of fetuin-A in type 1 DM patients with microalbuminuria. Furthermore, the role of valsartan as an inhibitor of renin-angiotensin-aldosterone system (RAAS) on serum levels of fetuin-A was assessed. The rationale of this study was to assess changes in fetuin-A as a possible predictor marker of endothelial dysfunction and its changes after inhibition of RAAS.

## MATERIALS AND METHODS

We randomly selected 32 eligible patients who were known cases of type 1 DM with confirmed

microalbuminuria. This cross-sectional study was performed from January 2010 to May 2011 in Isfahan, Iran. To select the participants, medical files of 270 type 1 DM patients who had been referred to Isfahan Endocrine and Metabolic Research Center were re-evaluated. Among them, 32 eligible patients who were candidates for receiving angiotensin receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors and met the inclusion criteria were included. The inclusion criteria were as follows: type 1 DM age of onset less than 15 (child-onset disease); glomerular filtration rate (GFR) equal to or greater than 90 mL/min/1.73 m<sup>2</sup>; normal blood pressure at three consecutive measurements (less than 95 percentile for age and sex); normal cardiovascular examination (approved by a single cardiologist); negative urine culture, and receiving no medication other than insulin.

Microalbuminuria was measured in 2 fasting urine samples with a sampling interval of at least 12 months.<sup>7</sup> The patients who had 2 abnormal results were included in the study. An albumin-creatinine ratio (ACR) equal to or greater than 30 mg/g was considered abnormal. Regarding microalbuminuria, the selected patients were candidates to receive ARBs or angiotensin-converting enzyme inhibitors as a part of the approved management to treat microalbuminuria.

The fasting urine samples were used for measuring ACR when blood glucose was in the acceptable range (fasting blood glucose less than 140 mg/dL and trace or negative urine dipstick results for glucose). Albumin was measured by enzyme-linked immunosorbent assay method on the fasting first morning urine sample. Blood samples were obtained to determine serum fetuin-A levels at time 0 (before starting the study), and 2 months after receiving the medication. Serum fetuin-A level was measured using a human fetuin-A enzyme-linked immunosorbent assay kit (BioVendor Laboratory Medicine, Brno, Czech Republic). The assay used a sandwich enzyme-linked immunosorbent assay technique with 2 polyclonal antibodies, which bind to different epitopes of human Fetuin-A. The standard used in the kit was a natural alpha 2-Heremans-Schmid glycoprotein (MW 49 kD) isolated from human blood. The intra- and inter-assay coefficients of variation were less than 5%. Valsartan tablets (Diovan, Novartis, Basel, Switzerland) with a single dose of 1 mg/kg/d up

to 80 mg/d was administered. This medication was selected because of extended half-life and the ease of administration.

In addition, serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), urea, creatinine, and hemoglobin A1c (Hb A1c) levels were determined 12 to 14 hours after fasting state. Glomerular filtration rate (GFR) was measured by the Modification of Diet in Renal Disease equation.

Written informed consent was obtained from all the participants before initiation of the study. The study was approved by local ethics committee, and carried out in accordance with the Declaration of Helsinki.

### Statistical Analyses

The data are reported as the mean  $\pm$  standard deviation. The SPSS software (Statistical Package for the Social Sciences, version 18.0, SPSS Inc, Chicago, Ill, USA) was used to perform statistical analysis. The data were tested for normality of distributions and homogeneity of variance. The paired Student *t* test was used to assess the significance of any change within groups. Linear regression analysis was used to evaluate correlation with all factors included in the model. The power of the study was 0.84. Statistical significance was accepted at a *P* level of .05.

### RESULTS

Thirty-two patients with child-onset type 1 DM were included. The mean age of participants was 21.65  $\pm$  0.38 years, with the median value of 19 years. The women and girls were predominant (21 females and 11 males). The mean values of Hb A1c, HDL, LDL, cholesterol, and triglyceride were not significantly different between the males and the females (Table 1). Furthermore, the mean values of fetuin-A before administration of valsartan were not significantly different between these groups (64.22  $\pm$  5.32 ng/mL versus 61.39  $\pm$  14.98 ng/mL respectively). The same was true after valsartan administration.

After administration of valsartan, the serum level of fetuin-A and urine ACR decreased significantly (Table 2). Before valsartan administration, the serum levels of fetuin-A did not have significant correlation with urine ACR or serum lipids. Nonetheless, a negative correlation was achieved between serum

**Table 1.** Clinical and Paraclinical Characteristics of the Participants

Characteristics	Female	Male	P
Body weight, kg	59.85 ± 11.98	61.75 ± 17.27	> .05
Height, cm	158.70 ± 10.13	163.25 ± 10.44	> .05
Body mass index, kg/m <sup>2</sup>	37.50 ± 5.86	37.76 ± 9.44	> .05
Mean blood pressure, mm Hg			
Before study	100.00 ± 12.35	107.00 ± 13.04	> .05
After study	92.00 ± 10.75	101.00 ± 10.27	.04
Serum triglyceride, mg/dL	100.95 ± 45.51	93.09 ± 47.74	> .05
Total serum cholesterol, mg/dL			
Before study	157.28 ± 38.23	153.27 ± 19.04	> .05
After study	154.03 ± 20.76	152.12 ± 22.39	> .05
High-density lipoprotein, mg/dL			
Before study	49.57 ± 12.63	48.00 ± 13.41	> .05
After study	49.60 ± 10.98	47.78 ± 9.54	> .05
Low-density lipoprotein, mg/dL			
Before study	87.85 ± 25.19	92.54 ± 13.10	> .05
After study	85.56 ± 33.50	94.65 ± 12.36	> .05
Hemoglobin A1c, %			
Before study	8.00 ± 2.14	7.53 ± 1.74	> .05
After study	8.02 ± 1.17	7.48 ± 2.60	> .05

**Table 2.** Markers of Endothelial and Kidney Function Before and After Valsartan Administration

Marker	Before Valsartan	After Valsartan	P
Fetuin-A, ng/mL	62.27 ± 12.73	20.83 ± 26.39	< .001
Urine albumin-creatinine ratio, mg/g	45.72 ± 18.48	15.12 ± 9.14	< .001
Serum creatinine, mg/dL	0.89 ± 0.07	0.91 ± 0.04	> .05
Glomerular filtration rate, ml/min/1.73 m <sup>2</sup>	94.21 ± 2.08	91.88 ± 6.48	> .05

fetuin-A level after valsartan administration and serum LDLC level ( $P = .007$ ,  $r = -0.507$ ). The same correlation was not achieved between fetuin-A levels after valsartan administration and other serum lipid components (total cholesterol and triglyceride). However, urine ACR before and after valsartan administration did not have significant correlation with Hg A1c, serum cholesterol, triglycerides, HDLC, and LDLC levels. According to multiple regression analysis, only LDLC cholesterol level was the negative predictor of serum fetuin-A after valsartan administration ( $P = .01$ , Table 3).

## DISCUSSION

In this study, we assessed the serum levels of fetuin-A in type 1 DM patients. To the best of our knowledge, it is the first study that evaluates changes in fetuin-A level after administration of ARBs in type 1 DM. Fetuin-A (formerly known as alpha 2-Heremans-Schmid glycoprotein) belongs to the cystatin superfamily of cysteine protease inhibitor, mostly synthesized in human hepatocytes.<sup>8,9</sup> Several functions have been proposed for this protein including acting as an acute phase protein, anti-inflammatory and anti-

**Table 3.** Adjusted Predictors of Fetuin-A Levels After Valsartan Administration

Parameter	Unstandardized coefficients		Standardized coefficients	
	B	Standard Error	Beta	P
Serum creatinine	7.92	46.33	0.07	.87
Fetuin-A before valsartan	0.55	0.40	0.28	.19
Glomerular filtration rate	0.26	0.29	0.32	.38
Low-density lipoprotein	-0.79	0.28	-0.54	.01
Microalbuminuria before valsartan	0.01	0.31	0.01	.97
Microalbuminuria after valsartan	0.40	0.50	0.17	.44
Hemoglobin A1c	0.96	2.60	0.08	.72

fibrotic activity, anti-apoptotic role, and negative regulator of vascular ossification-calcification.<sup>10-14</sup>

Various studies on different ethnic groups (Americans, Germans, and Chinese populations) demonstrated high levels of fetuin-A in type 2 DM.<sup>15-17</sup> Nonetheless, study on nondiabetic population with normal kidney function documented lower levels of fetuin-A in those with three-vessel coronary artery disease in comparison with normal population.<sup>18</sup> Regarding the different results obtained about the serum levels of fetuin-A under different conditions, Roos and colleagues proposed a biphasic association of this protein with vascular disease.<sup>3</sup> According to Roos and colleagues and the results obtained in similar studies, in nondiabetic and rather healthy diabetic patients without prevalent vascular disease, higher level of fetuin-A was associated with metabolic and vascular risks. The reverse result was achieved in patients with established vasculopathy.<sup>3,15,19</sup> In concert with these studies, Eraso and colleagues showed that type 2 DM patients with peripheral artery disease had lower levels of fetuin-A compared with those who did not suffer from peripheral artery disease.<sup>6</sup> However, Lorant and colleagues did not attain similar results.<sup>2</sup>

While there are controversial reports on serum concentration of fetuin-A in vasculopathies, the role of high fetuin-A levels in obese patients have been accepted widely.<sup>20-24</sup> Brix and colleagues showed a decrease in serum fetuin-A levels in morbid obese female after weight loss.<sup>21</sup> High levels of fetuin-A in addition to low levels of adiponectin and retinol binding protein-4 have been demonstrated in obese patients.<sup>22,23</sup> This is while adding ezetimibe to weight loss dramatically decreased serum levels of fetuin-A and retinol binding protein-4, and increased plasma adiponectin level.<sup>24</sup>

In type 1 DM patients, microalbuminuria has been known as an important marker of end-organ damage and may be used as an alternative marker of endothelial dysfunction.<sup>25,26</sup> In this study, we evaluated type 1 DM patients with normal or nearly normal GFR and microalbuminuria. Since there are conflicting data on fetuin-A concentrations in patients with low GFR levels, all patients with GFR less than 90 mL/min/1.73 m<sup>2</sup> were excluded from the study.<sup>27-29</sup> Furthermore, patients with confirmed vasculopathy or hypertension were not included. In these patients, microalbuminuria was the only sign of endothelial dysfunction. Previous studies have

been introduced microalbuminuria as a marker of early stage of diabetic nephropathy, medial arterial calcification, and endothelial dysfunction<sup>30-33</sup> In this study, the lowering effect of valsartan on fetuin-A concentration was accompanied by diminishing urine microalbumin level. We formerly showed that valsartan consumption decreased vascular cell adhesion molecule and increased nitric oxide levels (two more markers of endothelial function) in this group of patients.<sup>34</sup> In the current study, a significant negative correlation was achieved between fetuin-A levels after valsartan consumption and LDLC levels. According to the result of regression analysis, LDLC level was the only predictor of fetuin-A. Nonetheless, no association was attained between fetuin-A and albumin levels.

The atherosclerotic effect of lipids has been widely accepted. The results of a double-blind randomized placebo-controlled study by Aronis and colleagues revealed that walnut consumption affects apolipoprotein-A level, but not fetuin-A.<sup>35</sup> Furthermore, Hanefeld and colleagues demonstrated that lipid lowering agents did not lower fetuin-A levels in non-diabetic patients.<sup>36</sup> A recent review study by Mori and colleagues suggested that fetuin-A has a dual function in vascular diseases<sup>5</sup> They reported that existence of confounding factors such as diabetes mellitus and/or kidney dysfunction have been affected the results of various studies on fetuin-A.<sup>5</sup> We demonstrated that fetuin-A had a negative correlation with LDL. The role of LDL and lipid lowering agents were not studied in this study. However, the results showed that lipid lowering agents may have a role in fetuin-A levels in type 1 DM.

As mentioned above, only patients with nearly normal to normal GFR were included in the study. However, we did not demonstrate the association between LDLC and fetuin-A levels before valsartan administration. The parallel decreasing effect of valsartan on fetuin-A and urine microalbumin and the negative effect of LDLC on serum fetuin-A level after valsartan administration indicate that more attention should be paid to lowering the LDLC levels in type 1 DM patients.

In addition, according to the results of a large cohort study on elder population, high concentration of fetuin-A was associated with incident type 2 DM.<sup>37</sup> It should be investigated whether the lowering effect of ARBs may decrease the possibility

of diabetes occurrence besides its lowering effect on fetuin-A.

## CONCLUSIONS

We concluded that ARBs decrease fetuin-A levels and urine albumin levels concomitantly. Investigations are required to determine the long-term effect of ARBs on fetuin-A in type 1 DM. This finding should be interpreted with caution because of the study's noncontrolled design and a relatively small sample size due to its restricted inclusion criteria. The main strength of the study is its novelty in the type 1 DM patients.

## CONFLICT OF INTEREST

None declared.

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