Association between the development of diabetic foot and serum fetuin-A levels

Salim Özenç¹, Kemal Simsek², Ali O. Yildirim³, Erol Arslan⁴, Sebahattin Sari⁵, Mehmet Ince³, Oktay Sari⁴, Şirzat Yeşilkaya¹, Ümit Aydoğan¹, Halil Yaman⁶, Bayram Koç⁴, Kenan Sağlam⁴

¹ Gülhane Military Medical Academy, Department of Family Medicine, Ankara, Turkey
² Department of Undersea and Hyperbaric Medicine, Ankara, Turkey
³ Department of Emergency Medicine, Ankara, Turkey
⁴ Department of Internal Medicine, Ankara, Turkey
⁵ Department of Radiology, Ankara, Turkey
⁶ Department of Medical Biochemistry, Ankara, Turkey

Correspondence to: Erol Arslan, MD, Department of Internal Medicine, Gulhane Military Medical Academy, Ankara, Turkey, phone: +90-505-828-26-24, fax: +90-312-304-60-00, e-mail: earslan89@yahoo.com

Received: June 18, 2013.
Revision accepted: August 14, 2013.
PUBLISHED ONLINE: August 19, 2013.

Conflict of interest: none declared.

ABSTRACT

INTRODUCTION Fetuin-A is a glycoprotein secreted from hepatocytes, which affects diabetes and peripheral arterial disease. However, there has been no studies regarding the relation between diabetic foot and fetuin-A levels.

OBJECTIVES We aimed to analyze the association between diabetic foot development and serum fetuin-A levels.

PATIENTS AND METHODS Following the approval of the local ethical board, 137 patients were included in the study. Patients were divided into 3 groups: diabetes group (n = 49), diabetic foot group (n = 57), and control group (n = 31). In all patients, serum fetuin-A, C-reactive protein, magnesium, and hemoglobin A₁c (HbA₁c) levels were measured. Diabetic foot wounds were classified according to the Wagner classification and lower extremity arteries were evaluated by ultrasonography.

RESULTS Median fetuin-A levels in patients with diabetic foot were significantly higher than in those with diabetes. However, the differences in HbA₁c levels between both groups were not statistically significant. A positive correlation was found between the Wagner classification and ultrasound evaluation of the peripheral arteries (degree of atherosclerosis) in patients with diabetic foot. In the diabetic foot group, fetuin-A levels were also found to be positively correlated with ultrasound evaluation.

CONCLUSIONS We observed a positive correlation between serum fetuin-A levels and the development of diabetic foot.

INTRODUCTION Serum fetuin-A is a multifunctional glycoprotein, which is exclusively secreted from hepatocytes in humans.¹ It is also a potent systemic calcification inhibitor.² There is a genetic component of ectopic calcification in humans.³ However, in most cases, it is associated with other primary diseases such as atherosclerosis, cancer, or chronic renal failure, where calcification can be widespread and affect several organs.⁴,⁵

Previous studies have shown that decreased fetuin-A levels might be related to the development of vascular calcification and increased mortality in patients with severe renal failure.⁶,⁷,9 In a study by Ketteler et al.,⁵ low levels of fetuin-A were associated with cardiovascular mortality in patients on dialysis. Fetuin-A interacts with the insulin receptor tyrosine kinase and induces insulin resistance in rodents.¹⁰ An association between insulin resistance and type 2 diabetes in individuals with high serum fetuin-A levels was reported.¹⁰,¹⁵ Stefan et al.¹² showed that fetuin-A is an independent risk factor for developing diabetes in a prospective case-cohort study. Additionally, recent studies have emphasized that there may be an association between fetuin-A levels and peripheral arterial disease (PAD).¹⁷,¹⁸ Diabetic foot is one of the major complications of diabetes and is the main reason for nontraumatic major amputations. The common
clinical features of diabetic foot include ulcers, foot deformity, infection, neuropathy, PAD, osteomyelitis, and gangrene.\textsuperscript{19,20} The risk of lower-extremity amputation is higher in patients with diabetes and PAD than in those without diabetes.\textsuperscript{21} The role of fetuin-A and its involvement in patients with type 2 diabetes and PAD, who commonly suffer from advanced/systemic atherosclerosis, seems to be very complex and has not been fully understood as yet.\textsuperscript{21,22}

Although serum fetuin-A levels have been shown to be related to both diabetes and PAD, to the best of our knowledge, there have been no studies evaluating the relationship between serum fetuin-A levels and development of diabetic foot, which is one of the most serious consequences of PAD in diabetes.

The aim of this study was to investigate the possible relationship between serum fetuin-A levels and the development of diabetic foot.

\textbf{PATIENTS AND METHODS} A total of 137 patients were included in the study. Of those, 49 patients had type 2 diabetes without diabetic foot, 57 patients had type 2 diabetes with diabetic foot, and 31 subjects were healthy volunteers. All subjects were informed about the study procedure and they provided written informed consent. The study was approved by the local ethical committee of the Gulhane Military Medical Academy, Ankara, Turkey, in accordance with the Declaration of Helsinki.

Patients with type 2 diabetes were recruited from the Department of Endocrinology of the Gulhane Military Medical Academy. Type 2 diabetes was diagnosed according to the 2013 guidelines of the American Diabetes Association and patients with disease lasting 10 years or more were included.\textsuperscript{19} Patients with diabetic foot were recruited from the Department of Underwater and Hyperbaric Medicine and they were classified according to the Wagner classification.\textsuperscript{20} All patients in the diabetic foot group had diabetes for more than 10 years. The control group was recruited from the Department of Cardiology. Patients who had ischemic heart disease, advanced renal failure, or received dialysis treatment were excluded from the study. The diabetic group was fed on diet and received oral antidiabetic and insulin treatment. The diabetic foot group was fed on diet and received insulin treatment. There was no significant difference between both groups in terms of target hemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}) values.

In all patients, the anterior and posterior tibial arteries were assessed using a linear probe (12 MHz Logiq 9, GE Medical Systems, United States) by 2 radiologists at the same time, who had 8 and 10 years of experience in color Doppler ultrasonography. The plaque formations were divided into 3 groups according to the degree of calcification: mild, moderate, and severe. Calcification of the plaque of less than 25% and more than 75% was determined as mild and severe, respectively. The remaining plaque formations were considered moderate.

Fasting blood samples were collected after an overnight fast and the erythrocyte sedimentation rate (ESR) was determined in all subjects. Whole blood was collected in tubes without an anticoagulant for the preparation of serum. After clot formation, the serum fractions were obtained by centrifugation (2 000 x g, 10 min, 4°C). All serum samples were stored at −80°C until the assay. Serum HbA\textsubscript{1c}, C-reactive protein (CRP), ESR, and fetuin-A levels were assessed. Hypomagnesemia is known to be a common finding in patients with uncontrolled diabetes. It is associated with abnormal platelet action and development of neuropathy, both of which are risk factors for foot ulcers. Therefore, we also measured serum magnesium levels in our patients.

All assays were performed in the laboratories of the Gulhane Military Medical Academy Hospital, with an Olympus AU2700 autoanalyzer using its own kits (Olympus Diagnostics, Hamburg, Germany). ESR was measured using the Vacuette Automated ESR System (Greiner Bio-One GmbH, Frickenhausen, Germany). HbA\textsubscript{1c} levels were measured using high-pressure liquid chromatography (Thermo Electron, Thermo Finnigan, San Jose, California, United States) with a UV-Vis detector and using HbA\textsubscript{1c} kits (Recipe Chemicals – Instruments GmbH, Munich, Germany). Serum fetuin-A were measured using a human fetuin-A enzyme-linked immunosorbent assay (ELISA) kit (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic) in an ELISA plate reader (Synergy HT, Multidetection Multi-Plate Reader, Bio-tek Instruments, Inc., Winooski, Vermont, United States). The inter- and intraassay coefficients of variations were below 9%. Calibrators, kit controls, and serum samples were all added on each microwell with an incubation period of 30 min. After 3 washing intervals, 100 µl of an enzyme conjugate (peroxidase-labeled anti-CRP) was added on each microwell for additional 15-minute incubation in room temperature in the dark. The reaction was stopped with a stop solution, and photometric measurement was performed at the 450-nm wavelength. The amount of serum analyte was calculated as ng/ml with a graphic that was made based on the absorbance levels of the calibrators.

\textbf{Statistical analyses} Statistical analyses were performed using the SPSS for Windows V.15.0. Descriptive statistics were shown as frequencies and percentages for categorical variables and mean ± standard deviation or median (min–max) for continuous variables where appropriate. Distributions of continuous data were evaluated with the one-sample Kolmogorov–Smirnov test. The \(\chi^2\) test was used to compare sex distribution of the groups. Continuous variables were compared by the Kruskal–Wallis test, and the Bonferroni-corrected Mann–Whitney \(U\) test was used as a post hoc test. To evaluate
Data are presented as median (min–max).

a  χ² test,    b Kruskal–Wallis test

Abbreviations: BMI – body mass index, F – female, M – male

**TABLE 2** Fetuin-A levels and other blood parameters in control, diabetes, and diabetic foot groups (n = 137)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diabetes</th>
<th>Diabetic foot</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>magnesium, mg/dl</td>
<td>2.1</td>
<td>1.8</td>
<td>1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.4</td>
<td>7.7</td>
<td>7.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>13.8</td>
<td>11.3</td>
<td>18.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>14</td>
<td>23</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fetuin-A, ng/ml</td>
<td>36</td>
<td>85</td>
<td>123</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median.

a  Kruskal–Wallis test
b  Bonferroni-corrected Mann–Whitney U test (control vs. diabetes groups)
c  Bonferroni-corrected Mann–Whitney U test (control vs. diabetic foot groups)
d  Bonferroni-corrected Mann–Whitney U test (diabetes vs. diabetic foot groups)

Abbreviations: CRP – C-reactive protein, ESR – erythrocyte sedimentation rate, HbA1c – hemoglobin A1c

**TABLE 3** Wagner classification and ultrasound findings in the diabetic foot group (n = 57)

<table>
<thead>
<tr>
<th>Wagner classification</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>grade 1</td>
<td>13 (22.8)</td>
</tr>
<tr>
<td>grade 2</td>
<td>18 (31.6)</td>
</tr>
<tr>
<td>grade 3</td>
<td>13 (22.8)</td>
</tr>
<tr>
<td>grade 4</td>
<td>13 (22.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ultrasound findings</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild atherosclerosis</td>
<td>16 (28.1)</td>
</tr>
<tr>
<td>moderate atherosclerosis</td>
<td>15 (26.3)</td>
</tr>
<tr>
<td>severe atherosclerosis</td>
<td>26 (45.6)</td>
</tr>
<tr>
<td>total</td>
<td>57 (100)</td>
</tr>
</tbody>
</table>

**TABLE 1** Age, sex, and body mass index in control, diabetes, and diabetic foot groups (n = 137)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetes</th>
<th>Diabetic foot</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex, M/F</td>
<td>23/8</td>
<td>37/12</td>
<td>47/10</td>
<td>0.577a</td>
</tr>
<tr>
<td>age, y</td>
<td>51 (36–78)</td>
<td>59 (39–87)</td>
<td>64 (42–92)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>BMI</td>
<td>27.2 (22.4–34.4)</td>
<td>28.2 (22.1–34.4)</td>
<td>28.3 (22.0–34.5)</td>
<td>0.877b</td>
</tr>
</tbody>
</table>

Data are presented as median (min–max).

a  χ² test,    b Kruskal–Wallis test

Abbreviations: BMI – body mass index, F – female, M – male

**RESULTS** The study population comprised 107 men (78.1%) and 30 women (21.9%). The demographic features of the subjects are summarized in **TABLE 1**. Median fetuin-A levels of the patients with type 2 diabetes were found to be higher than those of the control group (85 ng/ml and 36 ng/ml, respectively, P = 0.026) and median fetuin-A levels of the patients with diabetic foot (123 ng/ml) were higher than those of the diabetic patients and the control group (P = 0.02 and P <0.001 respectively). The differences were statistically significant. The median HbA1c levels of the control, diabetes, and diabetic foot groups were 5.4%, 7.7%, and 7.6%, respectively. The statistical analysis showed no differences between HbA1c levels in the diabetes and diabetic foot group (P = 0.99). Moreover, there were no significant differences in serum magnesium levels between the diabetes and diabetic foot group. The median values of serum magnesium, HbA1c, CRP, ESR, and fetuin-A are presented in **TABLE 2**. Serum CRP, ESR, and fetuin-A levels were found to be significantly different between the diabetes and diabetic foot groups (**TABLE 2**). The differences were still observed after the groups were adjusted for age and body mass index (BMI). To evaluate the effect of diabetes on fetuin-A levels, a multivariate covariance analysis was performed, which showed that the diabetes status was significant after adjustment for age and BMI (P <0.001, R² = 0.229).

Blood pressure was similar in all groups. In the diabetic foot group, 22.8% of the patients had grade 1, 31.6% had grade 2, 22.8% had grade 3, and 22.8% had grade 4 diabetic foot according to the Wagner classification. The Wagner classification and ultrasound findings in this group are presented in **TABLE 3**.

A statistical analysis showed a positive correlation between the Wagner classification and ultrasound findings of the peripheral arteries (P <0.001). In the diabetic foot group, fetuin-A levels were found to be positively correlated both with the grade of diabetic foot (P <0.001) and ultrasound findings (P <0.001). The correlation between fetuin-A levels and Wagner grade and ultrasound findings is shown in **TABLE 4**.

**DISCUSSION** We found that serum fetuin-A levels of the patients with diabetic foot are higher the relationship between fetuin-A, Wagner score, and ultrasound findings, Spearman correlation coefficients were calculated. A covariance analysis was used as a multivariate analysis.
than those of the patients with type 2 diabetes or healthy individuals.

Our study has several limitations. First, the study group was small. Second, control subjects were younger than diabetic patients. To overcome this limitation, we performed a multivariate analysis and the difference in serum fetuin-A levels between the groups was still observed even after age correction. Nonetheless, our study is the first to report a relation between fetuin-A levels and diabetic foot. This finding will provide the basis for future research, which should distinguish between the ischemic, neuropathic, and mixed origin of diabetic foot to clarify the role of fetuin-A in the pathogenesis and treatment of this diabetic complication.

Lorand et al. found that serum fetuin-A levels in patients with type 2 diabetes and PAD were higher than those in patients with type 2 diabetes and without PAD. This is in line with our study, in which patients with diabetic foot had atherosclerotic lesions and calcifications of different degrees located in the lower extremity vessels. Our results are similar to those of Mehrotra et al., who reported that type 2 diabetic patients with coronary artery calcification had higher fetuin-A levels than those without calcification. Additionally, we found that fetuin-A levels were higher in patients with type 2 diabetes when compared with the control group. This finding is compatible with various previous studies that reported a positive correlation between fetuin-A and the development of type 2 diabetes.

The relation between nephropathy and serum fetuin-A levels is well documented. Therefore, we excluded patients with nephropathy. Our results showed that the prevalence of atherosclerotic lesions in lower limb peripheral arteries was positively correlated with serum fetuin-A levels. This finding is in contrast to the majority of the available studies that demonstrated a negative correlation between vascular calcifications and fetuin-A levels. However, those studies were performed in patients with renal disease and it is known that fetuin-A levels are decreased in renal disease. A number of studies reported that there were no significant differences in the levels of fetuin-A in patients on peritoneal dialysis because of chronic renal failure and in patients with degenerative aortic stenosis. Therefore, we think that our finding that shows a positive correlation between fetuin-A levels and lower limb arteriopathy may be due to our exclusion of patients with renal disease.

The effect of fetuin-A on the vascular structure is believed to be caused by two mechanisms. First, Eraso et al. reported that fetuin-A protects from ectopic and vascular calcification by increasing the solubility of serum calcium and phosphorus. Second, high fetuin-A levels have been shown to impair glucose metabolism. There are studies reporting that high fetuin-A levels are related to diabetes mellitus and coronary vascular disease. In those studies, fetuin-A was associated with an increase in insulin resistance and subclinical inflammation. Recent studies on the vascular effects of fetuin-A showed that high fetuin-A levels, rather than lower levels, were associated with greater risk of PAD, which is in line with our results.

The grades of diabetic foot according to the Wagner classification were found to be higher in patients with atherosclerotic lesions in our study. This finding is in line with the previous studies which reported that atherosclerotic PAD participated in the development of diabetic foot. This finding may be important for the development of treatment (hyperbaric oxygen therapy and amputation) or preventive modalities for diabetic angiopathy.

In line with the available literature, magnesium levels were found to be lower in patients with diabetes and/or diabetic foot compared with the control group. Furthermore, in patients with diabetic foot, the decreasing levels of magnesium showed a statistically significant correlation with increasing CRP and ESR levels. To the best of our knowledge, this finding has not been reported before.

In conclusion, according to our results, serum fetuin-A levels are higher in patients with diabetic foot. There is a need for further comprehensive studies with larger sample sizes and classification into different types of diabetic foot based on its origin in order to clearly demonstrate the association between serum fetuin-A levels and the development of diabetic foot. Moreover, the pathophysiology of the relation between fetuin-A levels and the development of diabetic foot needs to be clarified in future studies.

### REFERENCES


### TABLE 4 Correlation between fetuin-A levels and ultrasound findings and Wagner classification in the diabetic foot group (n = 57)

<table>
<thead>
<tr>
<th>Wagner classification</th>
<th>Fetuin-A</th>
<th>Wagner classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Ultrasound findings</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>r</td>
<td>0.935</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p</td>
<td>0.891</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Note:** The correlation coefficients (r) and significance levels (p) are shown for both fetuin-A levels and Wagner classification. The correlation is significant at the 0.001 level for both comparisons.
Association between the development of diabetic foot and serum fetuin-A levels

Związek między rozwojem stopy cukrzycowej a stężeniem fetuiny A w surowicy

Salim Özenç1, Kemal Simsek2, Ali O. Yildirim3, Erol Arslan4, Sebahattin Sari5, Mehmet İnce3, Oktay Sari1, Şirzat Yeşilkaya1, Ümit Aydoğan1, Halil Yaman6, Bayram Koç4, Kenan Sağlam4

1 Gülhane Military Medical Academy, Department of Family Medicine, Ankara, Turcja
2 Department of Undersea and Hyperbaric Medicine, Ankara, Turcja
3 Department of Emergency Medicine, Ankara, Turcja
4 Department of Internal Medicine, Ankara, Turcja
5 Department of Radiology, Ankara, Turcja
6 Department of Medical Biochemistry, Ankara, Turcja

Adres do korespondencji:
Erol Arslan, MD, Department of Internal Medicine, Gülhane Military Medical Academy, Ankara, Turcja, tel.: +90-505-828-26-24, fax: +90-312-304-40-00, e-mail: earslan89@yahoo.com

Praca wpłynęła: 18.06.2013.
Publikacja online: 19.08.2013.
Nie zgłoszono sprzeczności interesów.

SŁOWA KLUCZOWE
fetuina A, stopa cukrzycowa

STRESZCZENIE

WPROWADZENIE Fetuina A jest glikoproteiną wydzielaną przez hepatocyty, wpływającą na przebieg cukrzycy i chorób naczyń obwodowych. Dotychczas nie ma badań opisujących związek między stopą cukrzycową a fetuiną A.

CELE Celem badania była analiza zależności między rozwojem stopy cukrzycowej a stężeniem fetuiny A w surowicy.

PACJENTI I METODY Do badania zatwierdzonego przez lokalną komisję etyczną włączono 137 pacjentów, podzielonych na 3 grupy: chorych na cukrzycę (n = 49), chorych ze stopą cukrzycową (n = 57) i grupę kontrolną (n = 31). U wszystkich pacjentów oznaczono stężenie w surowicy fetuiny A, białka C-reakcyjnego i magnezu oraz poziom hemoglobiny A1c (HbA1c). Owrzodzenia w przebiegu stopy cukrzycowej klasyfikowano według Wagnera, a tętnice kończyn dolnych oceniano ultrasonograficznie.

WYNIKI Mediana stężenia fetuiny A u chorych ze stopą cukrzycową była istotnie większa, niż u pozostałych chorych na cukrzycę. Jednak poziom HbA1c w obu grupach nie różnił się znamieniowo. Wykazano dodatnią korelację między klasyfikacją Wagnera a oceną ultrasonograficzną tętnic obwodowych (stopnia zaawansowania miażdżycy) u chorych ze stopą cukrzycową. W grupie chorych ze stopą cukrzycową wykazano ponadto dodatnią korelację między stężeniem fetuiny A a oceną ultrasonograficzną.

WNIOSKI W niniejszym badaniu stwierdziliśmy dodatnią korelację między stężeniem fetuiny A w surowicy a występowaniem stopy cukrzycowej.