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The Effects of Insulin Therapy on Fetuin-A Levels in Type-2 Diabetic Patients

Tip-2 Diyabetik Hastalarda İnsulin Kullanımının Fetuin-A Düzeyi Üzerine Etkileri

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ABSTRACT

Aim: Fetuin-A is a natural inhibitor of tyrosine kinase activity and autophosphorylation of the insulin receptor. It is thought that fetuin-A plays an essential role in adjusting postprandial glucose level plays, insulin sensitivity, weight gain, and fat accumulation. In this study, we investigated the effect of insulin use on fetuin-A level in patients with type-2 diabetes mellitus. Also compared fetuin-a levels between healthy individuals and type 2 diabetes patients.

Material and Methods: The cross-sectional study was performed between May 2013 and July 2013. Sixty-nine patients with type 2 diabetes mellitus (37.7% oral antidiabetic agents and 62.3% insulin users) and 20 healthy individuals were included in the study. Diabetic group was divided into two subgroups as insulin group and oral antidiabetic agent group. We studied fetuin-A levels in these individuals. Additionally, we compared other biochemical parameters as ALT, AST, total cholesterol, LDL, HDL, VLDL, triglycerides, urea and creatinine in the diabetic group and control group as well diabetic year in subgroups of the diabetic group.

Results: The mean age in the diabetic group was significantly higher than the control group (54.97 ± 6.13 and 49.95 ± 8.82, respectively, p=0.025). ALT levels in the diabetic group were higher than the control group (p=0.018), but there was no statically significant difference between the two groups in terms of other biochemical parameters (p>0.05). Fasting blood glucose and HbA1c levels in the insulin group were significantly higher than the oral antidiabetic agent group (p=0.004 and p<0.001, respectively). Additionally, the diabetic year in insulin group was significantly higher in the insulin group than the oral antidiabetic agent group (p<0.001). The mean fetuin-A level was 65.5 ± 27.8 ng/mL in the control group and 86.3 ± 26.1 ng/mL in the diabetic group, as well fetuin-A levels were significantly lower in the control group than the diabetic group (p=0.003). Besides, the mean fetuin-A level was 88.6 ± 23.3 ng/mL in the oral antidiabetic agent group and 84.8 ± 27.8 ng/mL in the insulin group, but it was not statistically significant (p=0.570).

Conclusion: Fetuin-A level was significantly higher in the diabetic group, but there was no statistically significant difference between the oral antidiabetic agent group and the insulin group. Among the other biochemical parameters, only the ALT level was higher in the diabetic group than the control group, but this difference was not related to insulin therapy or oral antidiabetic agent. This is the first study to investigate the effect of treatment on fetuin-a levels in type-2 diabetic patients.

Key Words: Insulin, Fetuin-A, Oral antidiabetic agents

ÖZ

Amaç: Fetuin-A insülin reseptörünün otofosforilasyonunun ve tirozin kinaz aktivitesinin doğal bir inhibitörüdür. Fetuin-A'nın postprandiyal glikoz seviyesinin ayarlanmasında, insülin duyarlılığında, kilo alımında ve yağ birikiminde önemli bir rol oynadığı düşünülmektedir. Biz çalışmamızda, tip-2 diyabetes mellitus hastalarında insülin kullanımının fetuin-A seviyesi üzerine etkisini araştırdık. Aynı zamanda, sağlıklı gönüllülerle tip-2 diyabetes mellitus hastalarında fetuin-A düzeylerini de karşılaştırdık.

Gereç ve Yöntemler: Çalışma 2013 Mayıs ayı ile 2013 Temmuz ayı arasında yapıldı. Toplamda 69 tip-2 diyabetes mellitus'lu hasta (%37,7'sı oral antidiyabetik ajan kullanan, %62,3'ü insülin kullanan) ve 20 sağlıklı gönüllü dahil edildi. Diyabetik grup kendi arasında insulin kullanan grup ve oral antidiyabetik ajan kullanan grup olarak iki alt gruba ayrıldı. Bu çalışma grubunda fetuin-A düzeyleri bakıldı. Ek olarak; diyabetik grup ve kontrol grubunda ALT, AST, total kolesterol, LDL,HDL, VLDL, trigliserit, üre ve kreatinin gibi diğer biyokimyasal parametreler ve diyabetik grubun subgruplarında bunlara ek olarak diyabet yaşı karşılaştırıldı.

Bulgular: Diyabetik grupta yaş ortalaması, kontrol grubuna göre anlamlı olarak yüksekti (sırasıyla 54,97 ± 6,13 ve 49,95 ± 8,82, p=0,025). Diyabetik grupta kontrol grubuna göre ALT düzeyi anlamlı olarak yüksekti (p=0,018) ancak diğer biyokimyasal parametreler açısından her iki grup arasında anlamlı fark saptanmadı (p>0,05). İnsulin tedavisi alanlarda OAD kullananlara göre açık kan şekeri ve HbA1c düzeyi anlamlı olarak yüksekti (sırasıyla, p=0,004 ve p<0,001). Bununla beraber insulin grubunun diyabet yaşı OAD grubuna göre anlamlı olarak daha yüksekti (p<0,001). Ortalama fetuin-A düzeyi oral antidiyabetik ajan grubunda 88,6 ± 23,3 ng/mL ve insülin grubunda 84,8 ± 27,8 ng/mL idi, ancak gruplar arasında istatistiksel olarak anlamlı fark saptanmadı (p=0,570). Kontrol grubunda ortalama fetuin-A düzeyleri 65,5 ± 27,8 ng/mL olup diyabetik grupta 86,3 ± 26,1 ng/mL idi ve fetuin-A düzeyi kontrol grubunda anlamlı olarak düşüktü (p=0,003).

Sonuç: Fetuin-A düzeyi diyabetik grupta anlamlı olarak yüksekti ancak diyabetik grup içinde oral antidiyabetik kullananlarla insülin kullananlar arasında anlamlı fark saptanmadı. Karşılaştırılan diğer biyokimyasal parametrelerden ALT düzeyi diyabetik grupta fetuin-A ile benzer şekilde daha yüksekti ancak bu fark insulin kullanımı veya oral antidiyabetik kullanımı ile ilişkili değildi. Bu çalışma, tip-2 diyabetes mellituslu hastalarda tedavi rejiminin fetuin-A düzeyi üzerine olan etkisini araştıran ilk çalışmadır.

Anahtar Sözcükler: İnsulin, Fetuin-A, Oral antidiyabetik ajanlar

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease that is caused by impaired metabolism of carbohydrate, fat, and protein that resulting from problems about insulin release and/or the action of insulin; which has a high economic burden. According to the 2013 World Health Organisation's data there are approximately 247 million diabetics in the world. It is not different in Turkey. According to TURDEP II (Turkey Diabetes Mellitus, Hypertension, Obesity, and Endocrinologic Disorders prevalence Study) the frequency of diabetes mellitus in Turkish people is 13.7% (1). The most important cause of this increase is accepted as obesity, a decline in physical activity and population aging (2). Fetuin-A is a serum glycoprotein that is synthesized in the kidneys, choroid plexus and all major organs during fetal development, weighing approximately 60 kPa and is synthesized in the liver in adults (3,4). Fetuin-A reduces the rate of autophosphorylation of the insulin receptor and slows insulin signal transduction by binding to insulin receptor kinase and acting as an endogenous inhibitor in skeletal and adipocytes (5). Therefore, it suggested that fetuin-A molecule has an important role in regulating postprandial glucose level, insulin sensitivity, weight gain and fat accumulation (6). In this study, we investigated the effect of insulin use on fetuin-A level in patients with type-2 diabetes mellitus.

MATERIAL and METHODS

Ethical approval was obtained from the Haseki Training and Education Hospital Ethics Committee. All participants are volunteers and we received written approval form all of them. Sixty-nine patients with Type 2 diabetes mellitus were assigned into the diabetic group and twenty healthy individuals were assigned to the control group who applied to the Istanbul Haseki Training and Research Hospital diabetes polyclinic between May 2013 and July 2013 were included the study. The diabetic group divided into two subgroups according to the treatment protocols; as oral antidiabetic agent group and insulin group. Twenty-

six patients with a diagnosis of tip 2 diabetes mellitus for at least one year who received oral antidiabetic agent and forty-three patients with a diagnosis of tip 2 diabetes mellitus for at least one year who received insulin therapy were included in the diabetic group. The control group consisted of twenty healthy individuals. All patients and healthy individuals were between 35 to 65 years old. Patients with chronic renal failure, cerebrovascular disease, major trauma history within the last one year, peripheral arterial disease, decompensated heart failure, chronic hepatitis, proven coronary artery disease and chronic inflammatory disease were excluded. Following eight hours of the fasting period, blood biochemistry analysis including fasting blood glucose, urea, creatinine, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, alanine aminotransferase, aspartate aminotransferase, and HbA1c were carried out in all of the patients. Blood samples for fetuin-A were centrifuged at 2000g for 10 minutes after removing the flat dry tanks and waiting 30 minutes at room temperature. Serum was removed from the upper phase and stored at -80 ° C in eppendorf for further study. Fetuin-a measured using a commercial kit based on the principle of quantitative sandwich ELISA (Assaypro, USA, cat no: EG 63501-1) in the stored serum. Fetuin-a results were determined by reading ELX 800 UV model ELISA reader and calculated in ng/mL.

Statistical Analysis

SPSS 17 for Windows (Statistical Package for social sciences) was used to analysis all data obtained in the study. Descriptive statistics were expressed as mean and standard deviation of continuous variables, and categorical variables were expressed as number and percentage. Their distribution was evaluated by the Kolmogorov-Smirnov test. The comparison of differences in results between the patient and control group, which has a normal distribution, was performed using Student's t-test. Mann-Whitney U test was used in binary comparisons in non-normal distributions. Categorical variables were assessed by Pearson chi-square test. Pearson Spearman correlation test was used to compare two numerical data. Covariance analysis was performed to compare dependent variables. One-way ANOVA analysis was performed to compare multiple independent variables. $p<0.05$ or 95% confidence interval was considered statistically significant.

RESULTS

There were sixty-nine patients in the diabetic group consisting of twenty-two males and forty-seven females. The control group consisted of twenty healthy individuals, five males, and fifteen females. The average age was 54.97 ± 6.13 (male 53.86 ± 6.90 and female 55.49 ± 4.73) in the diabetic group and 49.95 ± 8.82 (male 49.60 ± 8.56; female 50.07 ± 9.20) in the control group. There was a statistically significant difference in age between the groups (p=0.025) as opposed to gender (p=0.555) Of the sixty-nine patients in the patient group, twenty-six used oral antidiabetic agent and forty-three used insulin therapy (oral antidiabetic agent 37.7%, insulin therapy 62.3%). One-way ANOVA analysis of the biochemical data of the diabetic group and the control group revealed a statistically significant difference in the fasting blood glucose, ALT and fetuin-A results of the groups (p<0.001, p=0.018 and p=0.003 respectively) (Table 1) There was a statistically significant difference between the subgroups of the diabetic group (insulin therapy group and oral

Table 1. Comparison of the biochemical parameters of diabetic group and control group

	Control (n=20)	Diabetic (n=69)	p
Fasting Blood Glucose (mg/dl)	95.00 ± 11.29	162.33 ± 62.36	**<0.001**
Urea (mg/dl)	27.05 ± 6.90	31.09 ± 12.03	0.156
Creatinine (mg/dl)	0.68 ± 0.16	0.70 ± 0.19	0.776
ALT (U/L)	19.75 ± 6.18	24.59 ± 11.99	**0.018**
AST (U/L)	20.95 ± 4.86	22.22 ± 8.01	0.387
Total cholesterol (mg/dl)	217.60 ± 73.19	205.03 ± 43.07	0.673
Triglycerides (mg/dl)	157.68 ± 85.58	181.97 ± 115.52	0.396
HDL cholesterol (mg/dl)	47.47 ± 10.46	47.30 ± 10.03	0.949
LDL cholesterol (mg/dl)	139.79 ± 53.03	123.52 ± 36.71	0.126
VLDL cholesterol (mg/dl)	31.05 ± 16.29	34.62 ± 18.37	0.445
Fetuin-A (ng/mL)	65.56 ± 27.84	86.30 ± 26.14	**0.003**

antidiabetic agent group) in terms of fasting blood glucose, HbA1c, and diabetes years (p=0.004, p<0.001 and p=0.01, respectively), but no statistically significant difference was found in terms of fetuin-A level (p=0.570) (Table 2). A covariance analysis comparing the fetuin-A averages between the control group and the diabetic group, under the influence of ALT effect, was performed and there was a statistically significant difference between the two groups (p=0.007). In the diabetic group ALT levels were significantly higher (p=0.018). In diabetic patients, there was no statistically significant relationship between fetuin-A and diabetes year, fasting blood glucose, HbA1c. There was also no statistically significant correlation between fetuin-A and ALT among the insulin treatment group and oral antidiabetic agent group. However, a statistically significant positive correlation was found when fetuin-A and ALT levels were compared in the entire diabetic group (p=0.037).

DISCUSSION

The number of diabetic individuals is increasing in parallel with the increase in the incidence of obesity and physical inactivity, population growth, aging, and urbanization. Fetuin-A is a natural inhibitor of tyrosine kinase activity and autophosphorylation of the insulin receptor. It is thought that adjusting postprandial glucose levels plays an important role in insulin sensitivity, weight gain and fat accumulation (6). In our study, we investigated whether the use of insulin in type 2 diabetes affected serum fetuin-A levels. We also compared fetuin-a levels between healthy individuals and type 2 diabetes patients. Song et al found that serum insulin levels and the insulin resistance homeostasis model evaluation (HOMA-IR) were positively correlated with serum fetuin-A levels. However, they have not found any relationship between impaired glucose tolerance (IGT) and serum fetuin-A levels. Interestingly, there was no relationship in terms of fetuin-a levels between healthy individuals and patients with impaired glucose tolerance (7). Based on these results, they suggested that serum fetuin-A was not effective in the development of impaired glucose tolerance from normal glucose homeostasis. Along with that, Stefan et al. found a positive correlation between fasting blood glucose values and serum fetuin-A levels in people who did not have diabetes (8).

In our study, we found a statistically significant difference as a result of one-way variance analysis between the control group and the diabetic group in terms of age, blood sugar, ALT and fetuin-A comparison of these groups. ALT levels were statistically significantly higher in diabetic patients (p=0.018) and fetuin-A levels were significantly higher in supporting the previous studies (p=0.003) (Table 1). Yılmaz et al. have detected high fetuin-A levels in adults with biologically proven nonalcoholic fatty liver disease (9). However, in a study conducted by Huang et al., high fetuin-a levels were found to be associated with the elevated fatty liver index, ALT and AST, and suggested that high fetuin-a levels could be an early indicator of nonalcoholic fatty liver disease (10). In our study, the ALT level was significantly higher in the diabetic group (p=0.018) (Table 1). This is probably due to the frequency of hepatosteatosis in diabetic patients. There was no statistically significant correlation between fetuin-A and ALT when the oral

Table 2. Comparison of the biochemical parameters of oral antidiabetic group and insulin group

	OAD (n=26)	Insulin therapy (n=43)	p
Fasting Blood Glucose (mg/dl)	134.85 ± 43.005	178.95 ± 66.672	**0.004**
Urea (mg/dl)	27.96 ± 6.78	32.98 ± 14.04	0.42
Creatinine (mg/dl)	0.723 ± 0.1861	0.684 ± 0.1975	0.416
Total cholesterol (mg/dl)	202.38 ± 40.529	206.63 ± 44.931	0.695
Triglycerides (mg/dl)	157.46 ± 57.853	196.79 ± 137.902	0.172
HDL cholesterol (mg/dl)	46.88 ± 9.101	47.56 ± 10.646	0.789
VLDL cholesterol (mg/dl)	31.12 ± 11.587	36.74 ± 21.314	0.220
LDL cholesterol (mg/dl)	123.38 ± 34.092	123.60 ± 38.596	0.981
AST	23.23 ± 7.845	21.60 ± 8.133	0.418
ALT	27.19 ± 13.997	23.02 ± 10.455	0.163
HbA1c (%)	7.216 ± 1.0354	8.886 ± 1.7048	**<0.001**
Diabetic Year	7.38 ± 4.989	11.07 ± 5.966	**0.010**
Fetuin-A (ng/dl)	88.619 ± 23.3534	84.898 ± 27.8658	0.570

antidiabetic agent group and insulin therapy group was compared. However, there was a statistically significant positive correlation between fetuin-A and ALT values in the entire diabetic group (p=0.037). Ou et al. reported that serum fetuin-A levels were elevated in patients with impaired glucose tolerance who didn't have non-alcoholic fatty liver disease compared to the control group (11). Similarly, in our study, a covariance analysis comparing the fetuin-A averages between the control group and the diabetic group, under the influence of the ALT effect, was performed and there was a statistically significant difference between the two groups (p=0.007).

According to our best knowledge, this is the first study that compares the fetuin-A levels among diabetic patients who is under the treatment of oral antidiabetic agent and insulin. Our study is the first from this point.

Consequently, Serum fetuin-A levels in diabetic patients were significantly higher than the control group; but there was no statistically significant difference between the oral antidiabetic agent or insulin users. Measurement of serum fetuin-A levels may be important for assessing the individual risk of type 2 diabetes in those with high risk for diabetes mellitus. However, we found that the treatment regimen does not have any effect on fetuin-A levels. But more studies are needed to generalize these results.

Ethics Committee Approval

Ethical approval was obtained from the Haseki Training and Education Hospital Ethics Committee:14/2013.

Conflict of Interest

No conflict of interest was declared by the authors.

Financial Disclosure

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Author Contributions

Concept: **Birsen Sahip, Utku Şentosun,** Design: **Birsen Sahip, Utku Şentosun,** Supervision: **Namık Yiğit,** Resources: **Muslih Ürün, Yonca Yılmaz Ürün,** Materials: **Muslih Ürün, Yonca Yılmaz Ürün,** Data Collection and/or Processing: **Muslih Ürün, Yonca Yılmaz Ürün,** Analysis and/or Interpretation: **Namık Yiğit, Muslih Ürün, Yonca Yılmaz Ürün,** Literature Search: **Muslih Ürün, Yonca Yılmaz Ürün,** Writing Manuscript: **Yonca Yılmaz Ürün, Muslih Ürün, Birsen Sahip,** Critical Review: **Yonca Yılmaz Ürün, Namık Yiğit,** Other: **Birsen Sahip, Utku Şentosun.**

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