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Original Article

Association of Serum Fetuin-A and Biochemical Parameters in Hemodialysis Patients

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ABSTRACT. Fetuin-A, a hepatic glycoprotein present in the circulation, is a potential inhibitor for systemic calcification. The main aim of this study was to evaluate the association between fetuin-A and other biochemical parameters as facilitator factors for developing atherosclerosis in hemodialysis (HD) patients. This case–control study was conducted on 44 HD patients undergoing treatment in 2012. Parathormone (i-PTH) and fetuin levels were performed by the enzyme-linked immunosorbent assay method, high-sensitivity C-reactive protein (hs-CRP) by chemiluminescence, low-density lipoprotein by direct enzymatic, calcium and albumin by colorimetric and phosphorous by ultraviolet (UV) methods. Chi-square was used for evaluating the association between variables and t-test was used for comparing the mean of the quantitative variables for the two groups. SPSS-16 software was used for data analysis and *P*-value less than 5% was considered as significant. Mean of serum fetuin level was 23.25 ± 4.90 ng/mL in HD patients and 32.92 ± 5.21 in the control group. Median of hs-CRP was 2.45 mg/dL in the patients and 1.00 mg/dL in the control group and i-PTH was 74.3 pg/mL in the patients and 7.30 pg/mL in the control group. The calcium–phosphorous product was 46.77 ± 14.22 mg/dL in the patient and 31.73 ± 6.48 mg/dL in the control group. A reverse significant association was found between fetuin-A and hs-CRP in this study. In this study, serum fetuin-A level in HD patients was lower than controls. Therefore, a low level of fetuin-A seems to be associated with atherosclerosis, inflammation and malnutrition.

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Introduction

Cardiovascular diseases are the most important causes of mortality among dialysis patients, and the mortality rate among dialysis patients is more than that of the general population.[1] Calcification is a pathologic process in

the patients with end-stage renal disease (ESRD) that results in a wide spectrum of pathological processes.[1] Atherosclerosis risk factors in the normal population are well known, but their role on uremic atherosclerosis is less studied.

Other than dialysis, duration and age, there are other known risk factors for calcification including elevated levels of parathormone (i-PTH) and serum C-reactive protein (CRP), dyslipidemia and hyper hemocysteinemia and reduction of serum albumin. Although hyperphosphatemia and increased calcium intake accompanied by increasing calcium phosphate product are important causes of calcification in ESRD patients,[2] recent findings reveal that inflammation also contributes to the development of calcification.[3] Inflammation is a multifactorial process in ESRD, and it seems that it is a resultant effect of common and uncommon risk factors.[3] CRP is an inflammatory marker that increases in the patients with acute inflammatory events. Association of CRP and cardiovascular events is very strong and remains independent even after adjusting for other risk factors.[4,5] In numerous investigations, the association of calcification with malnutrition, inflammation and atherosclerosis or related serum parameters such as fibrinogen elevation, CRP and hypoalbominemia have been confirmed.[6-8]

However, the high risk for cardiovascular diseases in ESRD patients may be related not only to increasing definitive risk factors but also to the relative lack of vascular protective factors. Fetuin-A, a hepatic glycoprotein present in circulation, is a potential inhibitor for systemic calcification that is found to decrease during inflammation.[9,10] Fetuin-A binds to calcium phosphate with severe adherence and, therefore, it acts as a buffer for serum calcium phosphate.

Various studies recommend that a decrease of the serum fetuin levels, which is observed in dialysis patients, facilitates extensive vascular calcifications, and these changes in vessel wall may be associated with increasing cardiovascular mortality. Other factors that seem to decrease calcification include PTH and related peptides.[11] High levels of PTH in circulation can lead to vascular calcification, but normal amounts of PTH have no influence on it or may inhibit it.

The main objective of this study was to evaluate the association, if any, between fetuin-A as an inhibitor factor for vascular calcification, atherosclerosis and biochemical parameters as facilitating factors for developing atherosclerosis in hemodialysis (HD) patients.

Subjects and Methods

In this case–control study, we randomly enrolled 44 patients on HD referred to our academic and treatment centers of the Gorgan Fifth Azar Hospital. Forty-four age- and sex-matched controls were selected among the healthy general population with normal urea and creatinine. People with active infections (fever or any evidence of infections), known malignancy, connective tissue disorders and inflammatory diseases were excluded from the study. Demographic information was extracted from patients' medical files. Before dialysis, 5 mL blood was obtained from the patients and, after isolating serums, they were stored at -70ºC in a freezer. The necessary biochemical tests were performed and, finally, the acquired information was statistically evaluated and analyzed.

Analysis of regression (logistic) was used for predicting an effect of increasing independent variables on the dependent variables, chi-square was used for evaluating an association between qualitative variables and t-test was used for comparing the mean of the quantitative variables for the two groups. SPSS software was used for data analysis and *P*-value less than 5% was considered as significant.

Results

In this study, 44 HD patients and 44 controls were evaluated and compared regarding serum fetuin-A level, calcium, phosphorous, PTH, CRP, albumin and LDL. Among the 44 HD patients, there were 24 males and 20 females. The mean age of the patients was 53.75 ±

12.83 years, whereas the mean age of the controls was 53.86 ± 12.28 years. In this study, the mean of serum fetuin level was 23.25 ± 4.90 ng/mL in HD patients and 32.92 ± 5.21 ng/mL in the control group. The mean serum albumin level was 4.07 ± 0.44 mg/dL in the patients and 4.46 ± 0.54 mg/dL in the control group. The mean serum LDL level was 82.4 ± 23.41 mg/dL and 96.87 ± 33.30 mg/dL in the HD and control groups, respectively. The median for hs-CRP was 2.45 µg/mL in the patients and 1.00 µg/mL in the controls; for PTH, the levels were 74.3 pg/mL in the patients and 7.30 pg/mL in the control group. The calcium–phosphorous product was 46.77 ± 14.22 mg/dL in the patients and 31.73 ± 6.48 mg/dL in the control group. The mean of dialysis duration was 29 months. A reverse significant association was found between fetuin A and hs-CRP in this study.

In addition, a significant association was found between fetuin A and albumin (P = 0.05). However, no significant association was found between fetuin with dialysis duration, PTH, LDL and calcium–phosphorous product.

Mean of serum fetuin-A level, albumin and LDL showed a statistically significant difference in the patients and healthy controls (P <0.001 for fetuin and albumin; P <0.02 for LDL). In addition, there was a statistically significant difference between serum phosphoric level and calcium–phosphorous product in both groups (P <0.001). However, there was no significant difference between the serum calcium levels in both groups (P = 0.18). Also, there was no significant difference between the two groups regarding age (P = 0.96).

For comparing the serum PTH level and hs-CRP in both groups, the median value was used because these parameters did not have a normal distribution. The median was 2.45 mg/dL for hs-CRP in the patients and 1.00 mg/dL in the controls. Median of PTH was 74.30 pg/mL in the patients and 7.30 pg/mL in the control group. Therefore, these differences between the serum PTH levels in the case and control groups had a statistically significant (P <0.001) difference, whereas this difference was not significant (P = 0.86) for the hs-CRP levels.

In this study, the mean serum PTH level was lower than the desired amount in 25 patients (56.8%), desired in five patients (11.4%) and higher than desired in 14 patients (31.8%). The calcium–phosphorous product was higher than normal in ten (22.7%) patients and lower than 55 in 43 (77.3%) patients. This product was lower than 55 in all the controls. Therefore, the calcium–phosphorous product showed a statistically significant difference between the case and control groups (P = 0.01, correlation co-efficient = 0.36). In this study, there was no statistically significant association between serum fetuin-A level with calcium, calcium–phosphorous co-efficient and LDL in the patients group. However, there was a statistically significant relationship between serum fetuin-A level and albumin in this group (P = 0.01, correlation co-efficient = 0.36). In our investigation, there was a reverse and statistically significant difference between serum fetuin-A level and hs-CRP in the patients (P = 0.004, correlation coefficient = -0.42), but there was no statistically significant association between fetuin-A and parathormone (P = 0.12) and dialysis duration (P = 0.6) in this group. In the control group, there was no statistically significant association between serum fetuin-A level with calcium (P = 1.00), calcium–phosphorous product (P = 0.89), LDL (P = 0.6) and albumin (P = 0.8). In the present study, there was a statistically significant association between serum fetuin-A level and hs-CRP in the control group (P = 0.05, correlation coefficient = -0.29). However, no statistically significant association was found between serum fetuin-A level and PTH (P = 0.77). There was a statistically reverse significant difference between serum fetuin-A level and hs-CRP in the patients (P = 0.004).

Discussion

It is now known that the high risk for cardiovascular diseases in ESRD patients, in addition to the conventional risk factors, may be related to a relative lack of vascular protective factors. Fetuin-A, a hepatic glycoprotein present in circulation, is a potential inhibitor

for systemic calcification and is found to decrease during inflammation.[9,10] Fetuin-A binds to calcium phosphate with severe adherence and, therefore, acts as a buffer for serum calcium phosphate and potentially can prevent calcification. Our study evaluated the fetuin-A levels with other parameters of calcification.

In the Kayser Caglar study conducted on 50 patients with chronic kidney disease in 2007, a reverse association was observed between serum fetuin-A concentration and hs-CRP.[12] In our study also there was a statistically reverse significant difference between serum fetuin-A level and hs-CRP in the patients ($P = 0.004$).

Angela Yee Moon Wang et al in a study on 238 HD patients in 2005 showed an important association between serum fetuin-A and MIAC syndrome (malnutrition, inflammation, atherosclerosis and valvular calcification) in the patients. In addition, a strong relationship was observed between IL-6 and CRP with fetuin-A in this study.[13] In 2005, Peter Stenvinkel and colleagues in a cohort study on 258 ESRD patients found an important reverse association between fetuin-A, CRP and IL-6.[14]

Ketteler et al[15] performed a study on 312 HD patients for evaluating calcification and cardiovascular disorders. They reported a reverse association between serum fetuin-A and CRP in these patients. Their results are completely in agreement with the recent Wang study.[13] In the Osamu Oikawa study in 2007 on 40 HD patients and 20 controls, it has been shown that the mean of serum fetuin-A in the HD patients had a negative association with hs-CRP. In addition, in this study, the fetuin-A concentration in HD patients was significantly lower than that in controls.[16] In the Jung et al investigation in 2008 on 222 HD patients, the authors showed that serum fetuin-A has a negative association with inflammatory markers in circulation.[17] In the Osamu Oikawa study, it was observed that there was a positive association between mean of serum fetuin-A level in HD patients with serum albumin.[16] In our study also there was a statistically significant association between serum fetuin-A level and albumin in the patients group ($P = 0.01$).

In the previous study of Wang, it has been shown that fetuin-A is well compatible with age-related CRP changes; also, the amounts of this acute phase protein do not have any association with dialysis duration.[18] In addition, in the prospective part of this study, it has been shown that treatment with dialysis is related to a mild but important decrease in serum fetuin-A levels. Ketteler and colleagues reported a higher degree of coronary calcification in the patients with long-term dialysis in comparison with short-term dialysis.[15] Furthermore, in the Osamu Oikawa study, it has been shown that the mean of serum fetuin-A in the HD patients group has a negative association with dialysis duration.[16] In our study, similar to Wang's study, there was no statistically significant association between fetuin-A and dialysis duration ($P = 0.6$).

In the Osamu Oikawa study, it was observed that serum fetuin-A levels in HD patients were significantly lower than that in the controls.[16] In our study also, the means of serum fetuin-A, albumin and LDL were significantly different in the patients and healthy control groups ($P < 0.001$ for fetuin and albumin, $P < 0.02$ for LDL).

Again in the Osamu Oikawa study, no important association was found between fetuin-A concentration and biochemical parameters such as Ca, P, iPTH and Ca–P product.[16] In our study, there was no statistically significant association between fetuin-A and PTH ($P = 0.12$), but there was a statistically significant difference between serum phosphorous levels and calcium–phosphorous product in both groups ($P < 0.001$).

In this study, the serum fetuin-A level in HD patients was lower than that in controls. Treatment with dialysis was related to a mild but important decrease in the serum fetuin level. The fetuin-A levels in the patients showed a positive association with serum albumin and a reverse association with hs-CRP. Fetuin-A, via its anti-inflammatory and calcification inhibition capacities, may play a regulatory role in atherosclerosis. An inflammatory reaction is the major cause of low fetuin level in the patients with malnutrition. Therefore, it is purposed that a low level of fetuin-A is associated

with atherosclerosis, inflammation and malnutrition. It seems that attempt on treatments for increasing fetuin-A and eliminating malnutrition in the patients will be helpful in preventing calcification among patients on HD.

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