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Evaluation of Serum Omentin Concentration and Its Association with Cardiovascular Risk Factors in Patients Undergoing Hemodialysis

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ABSTRACT

BACKGROUND AND OBJECTIVE: Inconsistent findings have been reported regarding the concentration of serum omentin in patients undergoing hemodialysis and healthy individuals. Omentin is correlated with several cardiovascular risk factors such as obesity, metabolic syndrome, atherosclerosis and inflammation. This study aimed to compare serum omentin concentration between hemodialysis patients and healthy subjects and determine its correlation with cardiovascular risk factors.

METHODS: This case-control study was conducted on 85 subjects divided into two groups of hemodialysis (n=55) and control (n=30). Serum omentin concentration and C-reactive protein (CRP) level were measured using ELISA and immunoturbidimetric method, respectively. Required data including age, cardiovascular risk factors, duration of dialysis, body mass index and history of cardiovascular diseases were collected via interviews and blood sampling and compared between groups.

FINDINGS: In this study, mean age of subjects in hemodialysis and control groups was 50.81±13.39 and 49.70±9.32 years, respectively (age range: 19-80 years). Serum omentin concentration was significantly higher in patients undergoing hemodialysis compared to control subjects (2.15±1.46 vs. 0.78±0.07 µg/ml, respectively) (p=0.0001). In addition, systolic blood pressure, blood glucose, creatinine and CRP were significantly higher in hemodialysis patients compared to control subjects, while levels of cholesterol, low-density lipoprotein, high-density lipoprotein and hemoglobin were significantly lower. Also, a significant positive correlation was observed between serum omentin and creatinine in all studied subjects (p=0.001, r=0.397). However, this correlation was not significant in patients undergoing hemodialysis. A positive correlation was found between serum omentin and blood glucose in the study population (p=0.001, r=0.590), and this correlation was less significant in hemodialysis patients (p=0.045 r=-244). Moreover, a significant negative correlation was observed between serum levels of omentin and triglyceride in hemodialysis patients (p=0.012, r=-244). No correlations were found between serum omentin and factors such as CRP, albumin, obesity and history of cardiovascular diseases or diabetes.

CONCLUSION: According to the results of this study, omentin concentration has a significant increase in patients undergoing hemodialysis compared to healthy subjects. In addition, serum omentin in these patients is positively correlated with a number of cardiovascular risk factors.

KEY WORDS: Omentin, Hemodialysis, Correlation, Cardiovascular risk factors.

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Introduction

Prevalence of renal failure leading to dialysis has been on a rising trend worldwide. Renal failure is considered as a major cause of mortality and disability among the patients (1). In addition, disorders such as atherosclerosis and chronic inflammation commonly appear in patients undergoing hemodialysis (2).

Inflammatory response causes changes in the serum concentration of proteins, and accurate measurement of serum protein levels could determine the presence and severity of these responses in order to predict cardiovascular complications (3). To do so, parameters such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and albumin are measured and evaluated. Other inflammatory markers could also be used for the assessment and follow-up treatment of inflammatory response in patients undergoing hemodialysis (4-6).

Evidence is suggestive of a significant association between inflammatory markers, such as CRP, and incidence of atherosclerosis, cardiovascular diseases and anemia in patients undergoing hemodialysis (7, 8). Recent studies have yielded conflicting results regarding the correlation between serum omentin, an adipokine derived from visceral fat, and conditions such as atherosclerosis and inflammatory response in patients undergoing hemodialysis (9). Accordingly, with its optimal anti-inflammatory properties, omentin could rectify the inflammatory response caused by tumor necrosis factor alpha (TNF-α) and ensure protection against carotid atherosclerosis (10, 11).

In research performed on animal models, omentin has been used for the treatment of cardiovascular diseases (12, 13). In some studies, increased serum omentin concentration has been shown to be associated with higher risk of cardiovascular diseases (14, 15). In a study in this regard, Alcelik et al. reported that omentin levels in patients undergoing hemodialysis were significantly higher compared to healthy control subjects (16). On the other hand, the results obtained by Tecke et al. indicated that serum omentin concentration was significantly lower in patients with chronic renal disorder compared to subjects in the control group (17).

Differences in the findings of these studies could be due to the variations in measurement methods or patient factors in terms of renal failure, body weight and insulin resistance (18, 19). Given the importance of cardiovascular complications in hemodialysis patients, determining the severity of inflammatory responses with appropriate parameters has been a major concern for medical researchers. Therefore, effective markers need to be used to identify high-risk patients and prevent cardiovascular complications. Several cardiovascular risk factors such as obesity, metabolic syndrome, atherosclerosis and inflammation are correlated with omentin (10, 11, 19-21). As a result, evaluation of serum omentin concentration in hemodialysis patients could contribute to the identification of high-risk cases.

This study aimed to compare the status of serum omentin concentration in patients undergoing hemodialysis with healthy subjects. Moreover, we investigated the correlation between serum omentin levels and inflammatory markers including CRP, albumin and cardiovascular parameters, such as abdominal obesity and diabetes, in hemodialysis patients.

Methods

This case-control study was conducted on 55 hemodialysis patients admitted to Shahid Beheshti Hospital of Babol, Iran and 30 healthy subjects as the control group. Study protocol was approved by the Ethics Committee of Babol University of Medical Sciences. Sampling was based on a difference of one microgram/milliliter in serum omentin levels in patients and control subjects with 80% test power and 95% confidence level.

Considering a standard deviation of 1.5, subjects were divided into two groups of 24. To compensate for patients who did not complete the study or had missing data, as well as to raise the study power, we doubled the number of hemodialysis patients and increased the number of control subjects by 25%.

Study population consisted of patients with end-stage renal disease undergoing hemodialysis three times a week for four hours using a membrane (USA Xenium Corp. Dialyzer, Baxter Healthcare, U.S). In addition, we enrolled healthy individuals with no particular medical conditions referring to laboratories of Shahid Beheshti Hospital of Babol (control group). Subjects in both groups were matched in terms of age and gender, and informed consent was obtained from all subjects prior to the study. Exclusion criteria of the study were as follows: 1) presence of malignancy and infectious diseases; 2) pregnancy; 3) diagnosis of hepatitis B and C; 4) collagen vascular diseases and 5) use of antibiotics. Anthropometric measurements including height, body weight, body mass index (BMI) and waist circumference were recorded in prepared checklists. BMI of subjects was calculated by dividing the body weight by the square of height (kg/m^2), and waist

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circumference was measured at umbilical level in centimeters. Systolic blood pressure was calculated using a sphygmomanometer for hemodialysis patients, and by obtaining samples from the right hand of control subjects in sitting position. In addition, 5 cc of blood was obtained for serum separation, and 2 cc of blood was collected from the patients in tubes containing ethylenediaminetetraacetic acid in order to determine hemoglobin, hematocrit and platelet count.

In hemodialysis patients, blood sampling was performed after one night of fasting prior to dialysis and 72 hours after their last dialysis session. Moreover, blood and urine samples were used to determine the levels of blood urea nitrogen (BUN), creatinine (Cr), blood sugar (BS), hemoglobin (Hb), hematocrit (Hct), total cholesterol (total-C), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin (Alb), uric acid (U/A), platelet count (Plt), CRP and serum omentin.

Blood samples were preserved in dry tubes and centrifuged for 10 minutes at 1000 g. Afterwards, samples were isolated, and frozen serum samples were analyzed simultaneously. Additionally, BUM, Alb and U/A were measured via the enzymatic colorimetric (GPO-PAP) method using Pars Test Kits (Iran) and Auto Analyzer Hitachi 902 (U.S.) in accordance with the instructions of manufacturers.

Level of Cr was measured without the elimination of proteins by GPO-PAP and Jaffe methods. This process was performed using Pars Test Kits and Auto Analyzer Hitachi 902 plasma glucose meter via glucose oxidase. Moreover, levels of Hb, Hct and Plt were measured using an automated cell counter (Sysmex Corp., U.S). For the point measurement of cholesterol, TG and HDL levels, we applied the GPO-PAP photometric method using Pars Test Kits and Auto Analyzer Hitachi 902 in accordance with the guidelines of manufacturers. Level of LDL was calculated using the Friedewald equation, and CRP level was quantified and measured using Auto Analyzer Hitachi 902 via immunoturbidimetric method. Required data of patients including age, gender, smoking habits, duration and cause of dialysis and history of diseases (e.g., diabetes, hypertension, hyperlipidemia and cardiovascular diseases) were recorded in prepared checklists.

Serum omentin measurement: Obtained venous blood samples were centrifuged for 10 minutes, and serum plasma samples were kept frozen. Omentin concentration was determined using the double-antibody enzyme-linked immunosorbent assay (ELISA) kits in accordance with the protocol of manufacturer. Moreover, omentin-1 was measured using China's biotec products and Hitachi 902 method in accordance with the guidelines of manufacturers. In this process, a microtiter plate was covered with specific antibodies of omentin-1, and serum or standard sample was added later. After incubation, samples were labeled with Ab and mixed with biotin, and eventually, combined with horseradish peroxidase. Following the incubation and rewashing of samples, chromogen was added in order to remove unbound enzymes. Color change was directly related to omentin concentration, and linear range varied between 0.50-64.0 mg/l. In this study, coefficient of variance for the level of intra-personal and interpersonal omentin was estimated at 4.4% and 3.2%, respectively.

Data analysis: In this study, data analysis was performed in SPSS V.20 (SPSS Inc., Chicago, Ill, U.S.). To determine normal distribution of data, we used the Kolmogorov-Smirnov test, and Chi-square test was used to analyze qualitative variables. In addition, correlation between studied variables was evaluated using the Spearman's correlation, and T-test was used for the comparison of qualitative and quantitative variables. Also, Mann-Whitney test was used to determine the difference between non-parametric data, and P-value of less than 0.05 was considered significant.

Result

This study was conducted on 55 hemodialysis patients and 30 healthy individuals (control group) with mean age of 50.81±13.39 and 49.70±9.32 years, respectively (Table 1). Main causes of renal failure among the studied patients were hypertension (36.4%), diabetes (23.6%) and polycystic kidney disease (9.1%), diabetes with hypertension (9.1%), infectious diseases (5.5%), urologic problems (5.5%), unknown causes (5.5%), nephrotic syndrome (1.8%), glomerulonephritis (1.8%) and preeclampsia (1.8%).

Serum omentin concentration was significantly higher in hemodialysis patients compared to control group (2.15±1.46 vs. 0.78±0.07 µg/ml, respectively) ($p<0.0001$) (table 1). In addition, patients in both groups were significantly different in terms of age, gender, smoking habits, systolic blood pressure and BMI. In hemodialysis patients, systolic blood pressure, BS, Cr and CRP were significantly higher, and levels of cholesterol, LDL, HDL and Hb were significantly lower compared to the control group. Other conditions observed in patients undergoing hemodialysis were as follows: hypertension (76.4%), diabetes (40%),

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hyperlipidemia (18.2%) and cardiovascular diseases (50.9%).

Table 1. Basic Data of Hemodialysis Patients and Control Subjects

Groups / Variables	Case N=55	Control N=30	Pvalue
Age (year) (Mean±SD)	51.42±15.21	49.70±9.32	0.5
Gender (Male/Female)	29.26	12.18	0.1
Smoking Habits(%)	7.3	1.2	0.8
Systolic Blood Pressure (mmHg)	130.38±15.46	110.18±14.7	0.02
Diastolic Blood Pressure (mmHg)	78.31±9.2	69.28±4.3	0.48
Waist Circumference (cm)	95.82±18.35	91.10±15.02	0.09
Body Mass Index (Mean±SD)	24.80±5.13	296.08±3.04	0.15
Hemoglobin (g/dl)	10.28±3.92	12.01±4.26	0.04
Platelet Count (104/µl)	196.40±82.10	221.28±9.11	0.63
Serum Creatinine (mg/dl)	8.17±3.50	0.89±0.19	<0.001
Blood Sugar (mg/dl)	190.09±72.55	92.27±10.46	<0.001
Cholesterol (mg/dl)	159.69±±37.30	197.17±41.86	<0.001
Low-density Lipoprotein Cholesterol(mg/dl)	94.35±32.29	129.68±36.06	<0.001

In hemodialysis patients, serum omentin had a positive correlation with Cr level; however, this correlation was not significant (P=0.063). It is noteworthy that this correlation was observed in all our subjects (P=0.001, r=0.397). In addition, a significant positive correlation was observed between blood glucose and omentin levels in both study groups (P=0.001, r=0.323). In hemodialysis patients, there was a significant correlation between blood glucose and serum omentin concentration (P=0.012, r=0.304). However, serum omentin level was higher in diabetic patients compared to non-diabetic subjects, and this difference was not significant (P=0.063). Other results of this study were indicative of a significant negative correlation between omentin and cholesterol levels in both groups (p=0.001, r=0.323). On the other hand, there was a significant negative correlation between TG and omentin levels in hemodialysis patients (p=0.045, r=-0.244). Finally, no correlations were found between serum omentin and parameters such as CRP, Alb, obesity, hypertension and cardiovascular diseases.

Discussion

According to the results of this study, serum omentin levels were significantly higher in patients undergoing hemodialysis compared to control subjects. In addition, omentin was observed to have a significant positive correlation with blood glucose, as well as a significant negative correlation with serum TG.

In the present study, no association was found between serum omentin concentration and cardiovascular risk factors, such as hypertension, hypercholesterolemia and obesity. However, there was a negative correlation between omentin levels and serum TG, as well as a positive correlation between serum omentin and BS levels. It is also noteworthy that no correlations were observed between inflammatory markers, such as CRP and Alb, and serum omentin concentration. Identification of inflammatory response is crucial in different medical conditions since inhibition of these responses is likely to prevent or decelerate disease progression. Inflammatory responses have been reported in several chronic diseases, such as chronic bronchitis and even knee osteoarthritis (22-24). To date, few studies have compared serum omentin levels between patients undergoing hemodialysis and healthy individuals. Our findings differ from previous studies conducted in this regard, while in some cases, they confirm some of their results. For instance, Alcelik et al. stated that serum omentin levels in hemodialysis patients were significantly higher than control subjects, which corresponds with the findings of the current study. However, the positive correlation observed between serum levels of omentin and Cr was not statistically significant, which is inconsistent with the results obtained by Alcelik et al. (16).

In another study, Tekce et al. compared healthy control subjects and patients with renal failure (with and without diabetes) in terms of serum omentin concentration. Unlike the present study, they reported that omentin levels were significantly lower in the patients compared to control subjects. As such, reduced omentin levels were attributed to other factors such as diabetes, malnutrition and inflammation (17). In another research, non-dialysis patients with chronic renal failure were compared with control subjects in terms of serum omentin levels and were reported to have higher serum omentin concentration, which is consistent with the findings of the present study (25). Several factors are

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considered to affect serum omentin levels, including obesity, diabetes, malnutrition, metabolic syndrome, atherosclerosis, inflammatory response and cardiovascular diseases (10,19,20).

In patients with chronic renal failure, especially those undergoing hemodialysis, events such as inflammatory response, atherosclerosis, infection and malnutrition are likely to occur, which are associated with reduced serum Alb and increased CRP (5,6,8). Reduction of serum Alb in dialysis patients could be a predictor for anemia, as well as lack of response to treatment (26). Diabetes is considered as a major cause of renal failure and is associated with reduced omentin concentration and insulin resistance (19).

Variations in the findings of studies conducted in this regard could be due to the presence of different factors in hemodialysis patients. Since it is able to prevent metabolic syndrome, omentin could have a remarkable effect on obesity, inflammation and cardiovascular complications (19,21,25). Prevalence rate of metabolic syndrome is relatively high in our country; therefore, evaluation of plasma omentin levels should be prioritized in medical schedules, especially in patients undergoing hemodialysis (27). It is noteworthy that patients with increasing insulin serum levels are exposed to lower risk of complications. According to the results of the present study, serum omentin levels were higher in diabetic patients, while in previous studies, omentin levels have been reported to be lower in diabetic patients compared to control group (16, 25). This difference could be due to increased levels of serum Cr in diabetic patients in our study.

A positive correlation has been reported between serum omentin and Cr levels in our research and the study performed by Alceik et al. (16). As a result, it is expected that hemodialysis patients with high Cr levels have higher omentin levels compared to control subjects. By contrast, no association was observed between serum omentin levels and cardiovascular risk factors, such as hypertension and obesity, in the current study. This could be due to the variations in factors such as age and obesity between patients with and without cardiovascular conditions since these factors largely affect analysis results. Due to lack of adequate physical activity, hemodialysis patients with cardiovascular diseases may remain asymptomatic. Therefore, actual number of cardiovascular patients undergoing hemodialysis may surpass the rate reported in the present study.

This study had some limitations. Firstly, this was a case-control study, and we could not precisely determine the cause and effect relationship between variables. Moreover, some of the parameters associated with omentin, such as CRP, could not be evaluated due to the small sample size. For another thing, a large number of patients with diabetes and hypertension were receiving treatment, and therefore, analysis results associated with BS, blood pressure and serum omentin required further investigation.

It is noteworthy that the actual population of patients with diabetes and hypertension may differ from the figures reported in the present study. Overall, our findings indicated that serum omentin concentration is significantly higher in patients undergoing hemodialysis compared to healthy individuals. Furthermore, serum omentin levels were positively correlated with blood glucose and negatively correlated with TG levels. However, given that a variety of factors affect serum omentin levels, finding an independent correlation between serum omentin and renal failure requires further investigation on a larger sample size. It is also recommended that future studies be conducted on the anti-inflammatory effects of omentin on patients with renal failure.

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