

CLINICAL STUDY

Relationship between Insulin Resistance and Inflammation Markers in Hemodialysis Patients

Ali Borazan

Mustafa Kemal University, Department of Nephrology, Hatay, Turkey

Dogan Nasir Binici

Erzurum Education and Research Hospital, Department of Internal Medicine, Erzurum, Turkey

Objective. The prevalence and risk factors of cardiovascular disease (CVD) are increasing in end stage renal disease (ESRD) patients. In this study, we sought to research the relationship between the insulin resistance, which is one of the risk factors for CVD, and the inflammation markers, especially C-reactive protein, fibrinogen, uric acid, and homocysteine levels in our patients who were recently diagnosed with ESRD and started hemodialysis. **Materials and methods.** 64 HOMA-IR-positive and 114 HOMA-IR-negative patients were enrolled in this study. Blood samples were obtained from the patients for fasting plasma glucose, insulin, CRP, fibrinogen, uric acid, total homocysteine, urea, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total protein, and albumin analysis after physical examinations and anamnesis were completed. **Results.** Fibrinogen and CRP levels of HOMA-IR-positive HD patients were significantly increased compared to non-insulin resistants. Furthermore, there is significant positive relationship between insulin resistance and serum CRP and fibrinogen levels in these HOMA-IR-positive HD patients ($r = 0.258$, $p < 0.001$). **Conclusion.** We found out that the fibrinogen and CRP levels are significantly high in HOMA-IR positive HD patients, according to determine the risk ratio for coronary artery disease in HD patients, and think that an assessment of insulin resistance is necessary.

Keywords hemodialysis, insulin resistance, C-reactive protein, fibrinogen, uric acid, homocysteine

INTRODUCTION

Cardiovascular diseases are the most common cause of mortality among end stage renal disease patients regardless of their primary renal disease, age, sex, race, and ethnicity. Cardiovascular disease-related mortality ratio among end stage renal disease patients is higher than the general population for all age groups.^[1]

Atherosclerosis is now recognized as an inflammatory disease. Exposure of the vascular endothelium to elevated blood lipids, smoking, hypertension, diabetes, and probably elevated plasma homocysteine or infectious micro-organisms leads to an endothelium-mediated inflammatory response characterized by increased vascular permeability, a procoagulant response, and formation of vasoactive molecules, cytokines, and growth factors. If the inflammatory response does not neutralize or remove the deleterious agent, it becomes persistent and is associated with progressive atheromatous changes.^[2]

The cause of insulin resistance is not completely understood either. Genetic and environmental factors may play a role, as do obesity and physical disability.^[3] Clinical and epidemiological studies showed that insulin resistance can be associated with inflammatory factors, and it is also shown to be a risk factor for type 2 diabetes, cardiovascular, and renal disorders. It has been demonstrated that insulin resistance can coexist with hypertension, increased serum triglycerides and small dense LDL levels, increased plasminogen activator inhibitor-1, and decreased levels of HDL as well.^[4]

Studies have also shown that individuals who had insulin resistance has increased levels of serum C-reactive protein (CRP), blood cell count, uric acid, and ferritin,^[5–7] though the relationship between HOMA-IR and inflammation is not clearly understood in HD patients.

In this study, we aimed to compare the relationship between HOMA-IR and inflammatory markers like CRP,

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Address correspondence to Ali Borazan, Mustafa Kemal Universitesi, Tip Fakultesi, Nefroloji Bilim Dalı, Hatay, Turkey; E-mail: borazanali@hotmail.com

fibrinogen, uric acid, and homocystine in HOMA-IR-positive HD patients.

MATERIALS AND METHODS

The present study was conducted in patients with end stage renal disease undergoing HD as renal replacement therapy after obtaining approval of the local ethics committee. A total of 178 patients (98 female, 80 male) were included in the study. Patients with liver, thyroid, parathyroid, and adrenal insufficiencies; receiving hormone replacement therapy; or having a history (within the last three months) of an acute event (e.g., cerebral or coronary), acute infection, major trauma, or surgery requiring anaesthesia were excluded from the study, as well as those who did not provide informed consent. Participants were instructed to fast for the 12 hours prior to study initiation. Demographic and clinical data such as age, gender, health status, and current medications were recorded.

All HD patients had a dietary intake of 30–35 kcal/kg/day, including 1.2 g/kg/day protein, 1000–1500 mg/day calcium, 600–700 mg/day phosphorus, and 200–250 mg/day magnesium. Certain patients were also receiving calcium acetate, vitamin-B complex, iron supplements, and erythropoietin as phosphorus-binding agents.

Demographic characteristics of patients (i.e., age, sex, BMI), hemodialysis durations, total/HDL/LDL cholesterol levels, triglyceride levels, and total protein and albumin values were collected using the medical records of patients admitted to our hospital. Blood samples were taken at hours between 8:00 and 10:00 am for fasting plasma glucose, CRP, fibrinogen, uric acid, and total homocysteine levels.

Laboratory Methods

Plasma glucose was measured using the hexokinase enzymatic reference method on a Roche Cobas Integra 800 Analyzer (Mannheim, Germany). Fasting plasma glucose ≥ 7.0 mmol/L or Oral Glucose Tolerance Test (OGTT) second hour plasma glucose ≥ 11.1 mmol/L was defined as DM, fasting plasma glucose ≥ 6.1 mmol was defined as impaired fasting glucose, and OGTT second hour plasma glucose ≥ 7.8 –11.0 mmol was defined as impaired glucose tolerance. Serum insulin was measured by using an immunometric sandwich assay with Immulite 2000 (Bio DPC, Louisiana, USA).

Serum C-reactive protein levels were measured using latex-enhanced nephelometry (Behring Nephelometer Analyzer System, Behring Diagnostics, Somerville, New Jersey, USA). Normal cutoff value of <1.0 mg/dL was

used. Patients who have elevated serum CRP levels underwent laboratory (ESR, leucocytosis, etc.) and physical examination to determine if they have any indications of infections of various systems. Blood samples for CRP were obtained after 21 days from patients who have been treated for infection. Plasma fibrinogen was measured using enzyme assay methods with Coag-A-Mate XC Plus (Organon-Tecnika, Alamogordo, New Mexico, USA). A normal cutoff value of 175–400 mg/dL was used. Uric acid was measured with Hitachi 737 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana, USA), with a normal cutoff value of 7 mg/dL. Plasma homocysteine was assessed by the fluorescence polarization immunoassay (Abbott IMX Instruments, Chicago, Illinois, USA) method, where a normal cutoff value of 15 micromol/L was used.

Insulin Resistance

The estimate of insulin resistance by HOMA (homeostasis model assessment) score was calculated with the following formula:

$$\frac{\text{Fasting serum insulin } (\mu\text{U/mL}) \times \text{fasting plasma glucose (mmol/L)}}{22.5},$$

as described by Matthews and coworkers.^[8] A ratio greater than 2.7 was defined as insulin resistance. With such a method, a high HOMA-IR score denoted low insulin sensitivity.^[8]

Statistical Analysis

Results were expressed as mean $\pm$ SD. Statistical comparisons were evaluated by computer using the SSPS (Statistical Package for Social Science) for Windows, Version 9.0 system (Release 9.0.0, standard version). Values between groups were compared using one-sided analysis of variance (ANOVA) test. The relationship between serum insulin resistance and CRP, fibrinogen and Hcy levels, and other factors was determined by Spearman's rho correlation test. The results are utilized in a 95% confidence interval; a *p* value less than 0.05 was designated as significant.

RESULTS

Of the patients who enrolled in this study 98 of 178 (55%) were female, 80 of 178 (45%) were male, and an average age of 46.3 ± 14.5 years (age interval 26–67) was found. The average age for 64 HOMA-IR-positive

cases (34 female, 30 male) and 114 HOMA-IR-negative cases (64 female, 50 male) were 45.9 ± 15.3 years (age interval 24–67) and 46.6 ± 14.2 years (age interval 20 ± 62), respectively.

The etiological causes of chronic renal failure cases were shown in Table 1. There has been no statistically significant relationship between HOMA-IR-positive and HOMA-IR-negative patients in terms of etiological causes ($p > 0.05$). The characteristics of HOMA-IR-positive and HOMA-IR-negative HD patients (e.g., age, sex, BMI) and average fasting plasma glucose, insulin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total protein, albumin, C-reactive protein (CRP), fibrinogen, uric acid, and homocysteine levels were shown in Table 2. Although there was no significant difference between two groups in terms of age, sex, total protein, triglyceride,

albumin, uric acid, BMI, and dialysis duration ($p > 0.05$), serum fibrinogen and CRP levels were found to be significantly higher in the HOMA-IR-positive group than the HOMA-IR-negative group ($p < 0.0001$).

A significant positive correlation was found between HOMA-IR, CRP, and fibrinogen in the HOMA-IR-positive group ($r = 0.258$, $p < 0.001$); otherwise, no relationship was found between the other parameters in HOMA-IR-negative patients.

DISCUSSION

This study is important because it is the first study that evaluates the relationship between HOMA-IR, CRP, fibrinogen, uric acid, and homocysteine in HOMA-IR-positive HD patients.

Insulin is the principal hormone of metabolic regulation. Insulin resistance is a change in physiologic regulation such that a fixed dose of insulin causes less of an effect on glucose metabolism than occurs in normal individuals.^[9] Insulin resistance reflects defective insulin action predominantly in skeletal muscle and liver.^[10]

In this study, CRP levels were found over the normal limits both in HOMA-IR-positive and -negative groups. The CRP levels were found significantly higher in the HOMA-IR-positive group vs. the HOMA-IR-negative group. In addition, a positive correlation between HOMA-IR and CRP was found.

Insulin resistance can be assessed by several methods, all of which estimate the relationship between plasma glucose and plasma insulin. Euglycemic-hyperinsulinemic clamp method is accepted as the gold standard for determining peripheral insulin resistance.^[11] Currently, the most commonly used method in clinical practice, because of its simplicity, is the HOMA-IR model. Homeostatic model assessment is a method for assessing β -cell function and insulin resistance from basal (fasting) glucose and insulin.^[12] The HOMA-IR model compares favorably with other models and has the advantage of requiring only a single plasma sample assayed for insulin and glucose. Nonetheless, HOMA-IR is highly correlated with insulin resistance measured using the euglycemic-hyperinsulinemic clamp method and is more suitable for population studies.^[12,13] The HOMA-IR model used in this study has been validated and widely used for determining the degree of insulin resistance in epidemiological studies.^[14]

There are reports that show that insulin resistance is not only associated with metabolic syndrome components like diabetes, dyslipidemia, hypertension, central obesity, and microalbuminuria, but also it is associated with hyperuricemia, hyperleptinemia, endothelial dysfunction, inflammatory status, thrombophilia, oxidative stress, and

Table 1
Primary diseases of the patients

Primary diseases	HOMA-IR (+)	HOMA-IR (-)
Diabetic nephropathy	14	28
Cronic glomerulonephritis	13	22
Hypertensive nephropathy	11	21
Cronic pyelonephritis	5	8
Obstructive nephropathy	4	7
Polycystic renal disease	4	7
Amyloidosis	3	5
Etiology unknown	10	16
Total	64	114

Table 2
Mean values of clinical and biochemical values of the groups

Clinical and biochemical values	HOMA-IR (+)	HOMA-IR (-)	p value
Age (years)	45.9 ± 15.3	46.6 ± 14.2	NS
Gender (female/male)	34/30	64/50	NS
BMI (kg/m^2)	24.8 ± 3.4	23.3 ± 3.8	NS
Dialysis time (months)	26.3 ± 11	25.6 ± 12	NS
Total cholesterol (mg/dL)	226.5 ± 38.4	214.6 ± 32.8	NS
HDL cholesterol (mg/dL)	35.6 ± 7.7	36.8 ± 8.9	NS
LDL cholesterol (mg/dL)	134.3 ± 40.1	122.6 ± 32.5	NS
Triglyceride (mg/dL)	232.8 ± 110.6	216.7 ± 98.8	NS
Total protein (g/dL)	7.44 ± 1.8	7.52 ± 1.9	NS
Albumin (g/dL)	3.26 ± 0.8	3.28 ± 0.7	NS
HOMA-IR score	4.2 ± 1.1	1.9 ± 0.5	<0.001
CRP (mg/dL)	13.8 ± 5.6	6.2 ± 3.5	<0.001
Fibrinogen	628.9 ± 186.7	468.6 ± 165.8	<0.001
Uric acid	8.4 ± 2.1	7.6 ± 2.8	NS
Total Hcy ($\mu\text{mol}/\text{L}$)	30.4 ± 11.4	28.7 ± 10	NS

non-alcoholic steatohepatitis of liver (NASH).^[13,15–20] In this study, a positive correlation was found independently between CRP and fibrinogen in HOMA-IR-positive HD patients.

C-reactive protein, produced in the liver in response to interleukin-6, has emerged as a marker of future cardiovascular risk among patients with stable and unstable angina.^[21] Recent evidence suggests that CRP may have direct pro-inflammatory effects.^[22]

According to the European Society of Hypertension-European Society of Cardiology report^[23] issued in 2003, CRP ≥ 1 mg/dL was added to the cardiovascular risk factors. There is much clinical evidence that shows that this protein has a predictive value as high as LDL cholesterol and is associated with metabolic syndrome.^[24,25]

Uric acid is a product of oxidation that is catalyzed by xanthine oxidase; during this reaction, free oxygen radicals are produced. Thus, it has been proposed that uric acid itself, regardless of renal function, is a marker of endothelial damage.^[26] In this study, uric acid levels are above normal in both groups, and there was no significant difference found between the two groups. Serum uric acid was positively associated with insulin resistance in several small studies.^[27] However, Clausen et al.^[28] reported that uric acid was not associated with insulin resistance after adjustment for confounding factors in young healthy Caucasians. Facchini et al.^[29] reported that the clearance of uric acid was decreased in patients with insulin resistance. However, in our study, no relationship was found between HOMA-IR and uric acid. We think that it is the reason that the uric acid levels were above normal in both groups; the patients in the control group are the HD-dependent patients, as well.

For decades, hyperfibrinogenemia has been recognized as a major risk factor for future cardiovascular events. Although several studies have clearly demonstrated strong associations of fibrinogen with most cardiovascular risk factors (e.g., age, obesity, cigarette smoking, diabetes, hypertension, dyslipidemia).^[30–32] In addition, plasma fibrinogen level is an inflammatory marker like CRP in cardiovascular events.^[33] The relationship between plasma fibrinogen levels and insulin resistance has been shown^[34]; similar to these studies, in our research, a positive correlation was found in HOMA-IR-positive HD patients between HOMA-IR and plasma fibrinogen levels, regardless of the other factors.

In this study, homocysteine levels were found above normal in both groups. However, there was no significant difference between groups. On the other hand, no relationship was found between HOMA-IR and homocysteine.

Elevated levels of homocysteine are accepted as an independent risk factor for cardiovascular diseases in the general population, which has been shown in previous

research.^[35,36] Also, it has been shown that serum homocysteine level is increased in inflammation, and that hyperhomocysteinemia induces inflammation.^[37] In experimental studies, it has been shown that hyperhomocysteinemia induces endothelial cell damage, smooth muscle cell proliferation, enhanced lipid oxidation, increase in platelet aggregation, modulation of leukocyte-endothelium interaction, and interference with clotting factors.^[38]

In conclusion, CRP and fibrinogen levels were found significantly elevated in HOMA-IR-positive HD patients vs. HOMA-IR-negative HD patients. On the other hand, a positive correlation was found between HOMA-IR and CRP and fibrinogen. Thus, to determine the risk ratio for coronary artery disease in HOMA-IR-positive HD patients, we think that an assessment of insulin resistance is necessary.

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