Association of interleukin-6 −174G/C promoter polymorphism with hypertension and left ventricular hypertrophy in dialysis patients

ATTILIO LOSITO, KAMINI KALIDAS, STEFANIA SANTONI, and STEVE JEFFERY

UO Nefrologia e Dialisi, Policlinico Monteluce, Perugia, Italy; and Medical Genetics Unit, St. George’s Hospital Medical School, Cranmer Terrace, Tooting, London, United Kingdom

Association of interleukin-6 −174G/C promoter polymorphism with hypertension and left ventricular hypertrophy in dialysis patients.

Background. Gene polymorphisms of proinflammatory cytokines, such as interleukin-6 (IL-6) and the chemokine receptor CX3CR1, have been found in association with cardiovascular disease in the general population. In dialysis patients, in whom the prevalence of cardiovascular comorbidity is strikingly high, these polymorphisms have not been investigated.

Methods. The −174G/C polymorphism of the IL-6 gene and the chemokine receptor CX3CR1 polymorphisms 249V/I and 280T/M were examined for their association with cardiovascular abnormalities in a cohort of 161 patients with end-stage renal disease (ESRD) treated by hemodialysis. Arterial blood pressure, electrocardiogram (ECG) ischemic changes, and left ventricular mass index (LVMI) were the parameters examined for the association study. The control group was made up of 169 healthy subjects.

Results. We found that for both IL-6 and chemokine receptor, genotype frequency and allelic distribution in both ESRD patients and controls were comparable. The genetic association study showed that in the whole group of dialysis patients, individuals with GC + CC genotype for the −174G/C polymorphism had a higher diastolic blood pressure (P = 0.008) and LVMI (P = 0.026) than GG homozygotes. The prevalence of left ventricular hypertrophy (LVH) in the former group was 58.6% vs. 39.2% in the latter (P = 0.02). The same analysis limited to diabetic patients in dialysis, showed that the prevalence of LVH in those with CG + CC genotype was 87.5% vs. 36.3% in those with GG genotype (P = 0.02). In diabetic patients, lower levels of serum albumin was found in the GC + CC genotypic group than in GG subjects; 34.63 ± 5.18 g/L vs. 41.75 ± 4.79 g/L (P = 0.003).

Conclusion. These data demonstrate an association between the IL-6 promoter polymorphism −174G/C and high blood pressure and LVH in hemodialysis patients, especially those with diabetes. The results strengthen the hypothesis that chronic inflammation is a mechanism of cardiovascular damage in dialysis patients and the role played by the IL-6 system in this mechanism.

Key words: hypertension, end-stage renal disease, polymorphism.

An association between changes in cytokine production and cardiovascular morbidity in uremic and nonuremic subjects has been suggested in several reports [1, 2]. In particular, for interleukin-6 (IL-6), where plasma levels in dialytic patients have been found to be abnormally elevated [3]. A polymorphism of the IL-6 gene (−174G/C) has been detected [4], and an association of this polymorphism has been found with coronary artery disease and high blood pressure in healthy men [5]. Recently, another component of this inflammatory system, the chemokine receptor CX3CR1, has been studied and it has been found that its genotype exhibits an association with susceptibility to cardiovascular disease [6]. Uremic patients display an abnormal prevalence of cardiovascular disease that cannot be fully explained by traditional risk factors, and alternative mechanisms, specific to uremia or dialytic treatment, have been put forward [7]. A link between chronic inflammation and cardiovascular risk in dialysis patients has therefore been proposed on the basis of experimental data, and recent reports suggest that increased levels of C-reactive protein predict overall cardiovascular mortality [8]. IL-6 is a primary determinant of hepatic production of C-reactive protein and plasma concentrations of IL-6 vary according to the genotype of the −174G/C polymorphism [5]. Several risk factors for hypertension and cardiovascular disease have been proposed in uremic patients, but the association of the IL-6 gene polymorphism with left ventricular hypertrophy (LVH) has not been investigated [9]. We undertook this association study with a dialysis cohort to ascertain whether the distribution of the polymorphism of the IL-6 gene and the chemokine receptor CX3CR1 in patients with end-stage renal disease (ESRD) suggest any association of these polymorphisms with cardiovascular disease.

METHODS

Study cohort

All patients (N = 161) treated by hemodialysis in our unit were enrolled in the study (Table 1). We had been
able to establish the cause of renal failure in 124 patients: chronic glomerulonephritis in 43 (26.8%), vascular disease in 23 (14.3%), diabetes in 22 (13.7%), nephrolithiasis, tubulointerstitial nephritis in 20 (12.5%), polycystic kidney disease in 10 (6.25%), and systemic disease [systemic lupus erythematosus (SLE), myeloma, and amyloidosis] in 6 (3.75%). In 36 (22.5%) patients the cause of renal failure remained undetermined. The average weekly time spent on dialysis was 11.99 ± 0.94 hours.

The dialysis fluid was a standard bicarbonate solution (Na 139 mmol/L, HCO₃ 34 mmol/L, K 2 mmol/L, Ca 1.75 mmol/L, Mg 0.75 mmol/L, Cl 108 mmol/L). Dialyzer surface was 1.3 to 2.1 m², according to the body surface. Dialysis membranes were made of polysulphone in 38%, of cuprophan in 37.5%, of AN69 in 9%, of polymethylmethacrylate in 7.6%, and of cellulose acetate in 6.9%. The vascular access was an arteriovenous fistula in 154 of 161 patients. A permanent intravenous catheter was used in the others.

Blood pressure was measured with a mercury manometer [10]. Hypertension was defined as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or above, or taking antihypertensive medication [11]. The average values of systolic and diastolic blood pressure taken in the first 4 weeks of the study (predialytic measurements of 12 sessions) are reported here and used in the analysis. Of 101 hypertensive subjects, 73 were on pharmacologic treatment. The following class of drugs were employed: β-blockers (N = 9), α-adrenoceptor antagonists (N = 9), calcium antagonists (N = 39), clonidine (N = 27), and angiotensin-converting enzyme (ACE) inhibitors (N = 24). Erythropoietin was used in 139 patients.

A standard 12-lead electrocardiogram (ECG) was performed during the first 4 weeks of the study. Ischemic changes in the ECG were classified according the Minnesota code criteria, and patients with signs of past myocardial infarction were included [12].

Echocardiography was performed in a nondialysis day about 24 hours after the dialysis session within the range of 8 weeks from the start of the study. Echocardiographic measurements were carried out according to the recommendation of the American Society of Echocardiography [13]. Left ventricular mass was calculated using the Devereux formula [14]. Left ventricular hypertrophy (LVH) was defined as a left ventricular mass indexed for body surface area (LVMI) greater than 125 g/m² in men or 110 g/m² in women. Body surface area was calculated according to the formula of Du Bois and Du Bois.

Dry weight was assessed clinically, with the aim of ensuring an edema-free state and a control of blood pressure without symptomatic hypotension. The interdialytic weight gain of the 11 interdialytic periods of the first month of the study was registered and the average value is reported and used in the study. Total body water was estimated by the formula of Chertow et al [15] specifically devised for the dialytic population.

The control group for genetic analysis comprised 169 healthy blood donors and hospital staff comparable for age and gender with patients (age, 61.9 ± 16.6 years; 105 men and 64 women). In these subjects the presence of cardiovascular disease had been ruled out by periodical health check-up. Controls and patients were residents in the same area and belonged to the same racial and ethnic groups.

**Laboratory measurements**

Venous blood samples were collected in the morning after an overnight fast on a mid-week dialysis day. Several hematologic, biochemical, and hormonal measurements were performed. For the present study, the following parameters are reported: hematocrit, serum albumin, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides.

The adequacy of dialytic dose was assessed, within 30 days from the start of the study, by the measurement of Kt/V calculated using the Daugirdas equation [16].

**Genetic analysis**

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The IL-6 G/C polymorphism was detected by PCR amplification using primers IL-6-1, 5'-TGACTTCAGCTTTACTCTTG-3' and IL-6-2, 5'-CTGATTTGAAACCTTATTAAG-3'. PCR was performed in a total volume of 25 µL [100 ng of genomic DNA, 1U Taq Polymerase, 25 pmol of each primer, 200 µmol of each deoxynucleoside triphosphate (dNTP), and 1.5 mmol Mg²⁺]. Thermocycling was performed under the following conditions: an initial dena-
turation at 95°C for 10 minutes followed by 35 cycles of 95°C for 45 seconds, 52°C for 45 seconds, and 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. Six microliters of the PCR product was digested with 10 U of the enzyme NlaIII in a 10 µL volume. The products of digestion were visualized in a 2.5% agarose gel stained with ethidium bromide.

The CX3CR1 gene polymorphisms were identified by the amplification of a 588 bp sequence using primers CX3CR1F, 5’-CCGAGGTCCCTTCAGGAAATCT-3’ and CX3CR1R, 5’-TCAGCATCAGTTTCAGGAA CTC-3’. The reaction was performed in a total volume of 25 µL (100 ng of genomic DNA, 1 U Taq Polymerase, 25 pmol of each primer, 200 µmol of each dNTP, and 1.5 mmol Mg²⁺). Thermocycling conditions were an initial denaturation at 95°C for 10 minutes followed by 35 cycles of 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. The T280M polymorphism was identified using the enzyme BsmBI and the V249I polymorphism was identified using PspI406I.

Statistical analysis

Data are reported as mean ± standard deviation (SD) or standard error (SE), as appropriate. The forward, stepwise logistic regression model was used to identify risk factors. The factors selected with this model were analyzed for their association with the chosen parameters, with odds ratio (OR) (95% CI) and chi-squared. The univariate analysis of variance was used to detect the difference on the means of the continuous variables, between subjects with different genotypes. Adjustment for covariates was done by multivariable tests. Comparison of adjusted means was done by appropriate tests. The data analysis was performed using the BMDB Statistical Software (release 7, Cork, Ireland).

RESULTS

Data were obtained for all but one patient, who was not thoroughly investigated and therefore excluded from the association study. Mean values of dialytic parameters were within the limits of National Kidney Foundation/Dialysis Outcomes Quality Initiative (K/DOQI) recommendation [17].

Hypertension was present in 101 patients (57 men, 44 women). Diastolic blood pressure was 80.5 ± 9.4 mm Hg in men and 75.7 ± 11.8 mm Hg in women (NS). Systolic blood pressure was 143.2 ± 20.4 mm Hg and 134.7 ± 24.7 mm Hg in men and women, respectively (F = 5.398, P = 0.021). Diastolic blood pressure was negatively correlated with age (t = 3.294, P = 0.001).

Ischemic changes on the ECG were found in 55 patients (30 men, 25 women). At the time of study inclusion, the prevalence of cardiovascular events was myocardial infarction in 17 cases (10.6%) and angina in 38 (23%).

LVMI was 146.8 ± 47.9 g/m² in the whole cohort, 150.9 ± 45 g/m² in hypertensive patients, and 138.5 ± 53 g/m² in normotensive subjects (NS). LVH was present in 47% of the examined population; in 33.3% of normotensive patients and in 54.3% of hypertensive patients (OR 2.380, CI 1.132 to 5.006; chi-squared 5.351; P = 0.02). In women, LVH was present in 40.6% (LVMI = 142.4 ± 43.7 g/m²) and in 52.5% of men (LVMI = 150.2 ± 50.9 g/m²) (NS). In diabetic subjects, the prevalence of LVH was 57.8% (LVMI = 160.5 ± 38.4 g/m²) and in nondiabetic subjects, it was 45.7% (LVMI = 144.6 ± 49.1 g/m²) (NS).

The multiple regression analysis was performed to detect the variables, from a set of traditional risk factors, with most influence on LVMI. Independent variables were the data of all patients, LVMI was the dependent variable. The stepwise procedure excluded the following variables: age, time on dialysis, and diastolic blood pressure. The selected predictor was systolic blood pressure (t = 2.175, P = 0.031).

Genetic polymorphisms

The distribution of polymorphisms of IL-6 (−174G/C) and chemokine receptor CX3CR1 (280T/M and 249V/I) genes was comparable between patients and controls (Table 2), and was in Hardy Weinberg equilibrium in both groups. The test was also performed on the subgroups (hypertensive and diabetic patients), which were also in accord with Hardy Weinberg.

Genetic association study

Age and time spent on dialysis were comparable between individuals with different genotypes: 67.0 ± 13.6 years and 98.4 ± 60.1 months, respectively, in patients with GC + CC genotype for the −174G/C IL-6 polymorphism vs. 66.8 ± 13.6 years and 90.8 ± 62.7 months, respectively, in those with the GG genotype. No association was found between ischemic changes on the ECG and any of the polymorphisms studied. A cardiovascular event was recorded in 34.8% of GC + CC subjects and in 39.1% of GG homozygotes (NS).
IL-6 polymorphism. CC homozygotes were not analyzed in the association study due to their small number, but were combined with the GC genotype group.

Serum albumin was 38.88 ± 4.48 g/L in patients with GC + CC genotype and 39.57 ± 4.94 g/L in GG individuals (NS). In diabetic GC + CC cases, serum albumin was 34.63 ± 5.18 g/L vs. 41.75 ± 4.79 g/L in GG subjects (Bonferroni test, \( F = 9.724, P = 0.003 \)). The body mass index was 24.6 ± 4.7 in GC + CC cases and 24.9 ± 4.3 in GG patients (NS).

Patients who were GC + CC for the −174G/C polymorphism of IL-6 had higher diastolic blood pressure and higher prevalence of LVH compared to those with GG genotype (Table 3). Diastolic blood pressure, adjusted for age and time in dialysis, was 85.0 ± 2.2 mm Hg (SE) in GC + CC individuals and 76.3 ± 1.0 mm Hg in GG homozygotes (difference = 8.7 mm Hg, 95% CI 1.211 to 12.614, \( P = 0.018 \)). In the 10 CC homozygotes, diastolic blood pressure was 79.9 ± 3.1 mm Hg (SE). The logistic regression (forward stepwise), with LVH as a dependent variable, and with age, gender, diabetes, albumin, hemoglobin, interdialytic weight gain, total body water, body mass index, blood pressure, and IL-6 polymorphism as covariates produced a model with the following predictors: systolic blood pressure \( (P = 0.026) \) and GC genotype \( (P = 0.04) \). The analysis limited to the CC genotype has not been performed as the small number of samples makes this statistically meaningless.

The prevalence of LVH in those with GC + CC genotype for IL-6 was 58.6% compared to 39.2% in GG subjects (OR 2.193; 95% CI 1.040 to 4.6414; chi-squared = 5.037; \( P = 0.025 \)). In hypertensive subjects, 66% of those with GC + CC genotype had LVH and 44% of GG patients (OR 2.544; 95% CI 1.086 to 5.952; chi-squared = 4.722; \( P = 0.03 \)). In those with the GC + CC genotype for the IL-6 polymorphism, the mean of LVMI adjusted for age, time on dialysis, total body water, serum albumin, and systolic blood pressure (recognized risk factors for LVH in dialysis patients) was 161.7 ± 7.0 g/m² (SE) compared to 139.2 ± 6.4 g/m² of GG individuals, mean difference 22.49 g/m² (95% CI 2.769 to 42.215; \( P = 0.026 \)).

In diabetic subjects, 40.9% had the GC + CC genotype, while 59.1% had the GG genotype. The distribution was comparable with the nondiabetics, 41.8% and 58.1%, respectively. The prevalence of LVH was 87.5% in the GC + CC group and 36.3% in those with the GG genotype (OR 2.406; 95% CI 1.055 to 5.488; chi-squared 4.968; \( P = 0.026 \)).

The analysis of the chemokine receptor polymorphisms \( (280M/T \) and \( 249V/I) \) showed no association between the individual polymorphisms and blood pressure or LVMI. Smoking habit, gender, body mass index, Kt/V, and type of dialytic membrane were analyzed together with polymorphism in the association study and were found to have no effect on hypertension or LVH.

### DISCUSSION

We found that the distribution of the polymorphisms of IL-6 and chemokine receptor CXCR1 in a cohort of unselected patients undergoing hemodialysis was not different from a control group of healthy subjects. The novel finding of this study is that patients with GC + CC genotype for the −174G/C polymorphism of the IL-6 gene had higher diastolic blood pressure and LVMI than subjects with the GG genotype. Together with hypertension, the C allele was a predictor of LVH. In diabetic patients, the C allele was also associated with lower serum concentration of albumin.

The small number of CC homozygote subjects has hampered the study of any association of this group with the above conditions, allowing conclusions only on the GC genotype. We are also aware that these findings come from a secondary analysis and therefore must be taken cautiously.

IL-6 is a circulating cytokine secreted by macrophages, lymphocytes, fibroblasts, adipocytes, and endothelial cells, in response to different types of inflammatory stimuli. IL-6 induces endothelial damage, stimulating the intracellular adhesion molecule-1 (ICAM-1) and enhancing the attachment and migration of leucocytes across the endothelial surface [18]. A clinical confirmation of this vascular effect comes from a very recent study in uremic patients where an association between increased levels of IL-6, *Chlamydia pneumoniae* seropositivity and progressive carotid artery stenosis has been found [19]. Also, hypertension may increase the risk of atherosclerosis via proinflammatory effects mediated by IL-6. A recent study in apparently healthy men found a strong
relationship between plasma levels of IL-6 and systolic and diastolic blood pressures [20].

The up-regulation of IL-6 found in dialysis patients is regarded as an important cause of morbidity and mortality, and has stimulated several investigations [21]. In most studies, IL-6 plasma levels have been found to be increased in ESRD patients treated with dialysis, while in a minority of patients normal or reduced values have been found [22, 23].

These variable results were first ascribed to technical problems and to the complex regulatory system of cytokines [24]. More recently, genetic studies have shown that a polymorphism in the promoter region of the IL-6 gene, a G/C change in position 174, influences plasma levels of IL-6 [5]. A gene-environment interaction has therefore been proposed to explain the blunted or the enhanced IL-6-mediated acute phase response, expressed by reduced or raised concentrations of IL-6 in different subjects and in different pathologic conditions. In healthy subjects, the C allele of the −174G/C polymorphism was associated with lower plasma levels of IL-6 [25]. Data on plasma IL-6 levels and −174G/C polymorphism in dialysis patients have not been reported in the literature.

Patients undergoing coronary artery bypass graft surgery had comparable baseline IL-6 levels across different IL-6 genotypes, but, after the procedure, the rise in IL-6 was greater in carriers of the CC genotype than in carriers of the G allele [26]. In the general population, the C allele is associated with higher levels of C-reactive protein whose gene expression is regulated by IL-6 [27]. These finding points to a difference in IL-6 production in response to pathologic stimuli that is genetically determined.

A possible confounding factor in our study was a survival effect. Although we believe that analysis of age and time on dialysis for individuals with different genotypes makes this an unlikely explanation, it cannot be definitely excluded. Furthermore, in our patients, the overall distribution of the IL-6 polymorphism was comparable with the control group. However, the different distribution of alleles within patient subgroups suggests a possible contributory role of IL-6 in some of the comorbid conditions found in dialysis patients. Hypertension has already been found in association with high levels of markers of inflammation such as IL-6 and ICAM-1 in otherwise healthy people, but not in renal patients [21]. Furthermore, a recent genetic association study suggested that the effect of the IL-6 −174G/C polymorphism on blood pressure is likely to be operating through an inflammatory pathway, more probably mediated by a local and not systemic production of IL-6 [5]. IL-6 is able to stimulate the central nervous system and the sympathetic nervous system, which may result in hypertension [28]. In rats treated with Nω-nitro-L-arginine methyl ester (L-NAME), hypertension was associated with increased expression of IL-6 in the vascular wall, the molecular plasticity of which was altered [29]. Of more clinical impact, and with some reference to our diabetic patients, is the recent report that has shown an association of IL-6 with insulin resistance and with hypertension in apparently healthy subjects [30]. Dialysis patients, in whom we found an association between this polymorphism and raised diastolic blood pressure, have several predisposing factors that may lead to activation of the inflammatory system, and these could act in synergy with the genotype. The inflammation-associated vascular damage in uremic patients has been well demonstrated [31]. The association of LVH with homocysteine concentration in ESRD raises the possibility of a connection between vascular damage and LVH [32]. The link between endothelial dysfunction and cardiac hypertrophy has been shown in untreated hypertensives in whom the drug-induced vasodilation was inversely correlated with the LVMI [33]. A suggestion that IL-6 may play a role in this complex mechanism comes from our finding of reduced albumin in diabetic patients with GC + CC genotype. The association of high IL-6 with reduced levels of albumin in dialysis patients is known to predict mortality in hemodialysis patients [34]. From the low levels of albumin in those with the GC + CC genotype, we can infer high IL-6 levels associated with this polymorphism. IL-6 also acts at the tissue level. In the experimental animal with early atherosclerosis, an increased expression of this cytokine in the fibrous plaque has been observed [35]. This indirect mechanism, mediated by endothelial dysfunction, may interact with a more direct effect of IL-6 on the myocardium, as has been shown in the experimental animal. One phenotypic characteristic of transgenic mice over expressing IL-6 and IL-6 receptor, is hypertrophy in adult myocardium, suggesting that IL-6–related cytokines regulate heart muscle cells and induce cardiac hypertrophy [36]. A new cytokine, cardiotrophin 1, with a structure closely related to IL-6, has been cloned, and the mRNA is expressed in the heart. This cytokine induces hypertrophy of cardiomyocytes [37]. These experimental findings also suggest that tissue IL-6, in addition to the circulating cytokine, may act on uremic heart cells in combination with hypertension and other factors, inducing LVH.

The combined effect of different polymorphisms, with putative inflammatory properties, has been studied in patients with asymptomatic carotid artery atherosclerosis. Subjects, homozygous for two polymorphisms, −174G of IL-6 and 6A of stromelysin-1, had an intima-media thickness in the carotid bifurcation greater than men who were homozygous for neither allele [38]. Finally, indirect support for our findings comes from the recent report of a protective effect from cardiovascular events of anti-inflammatory IL-10 genotype in dialysis patients [39].
CONCLUSION

Our results suggest that a genetic polymorphism of IL-6 is associated with high blood pressure and LVH in dialytic patients who also carry other risk factors for such complications. These results also support a role of the IL-6 system in the cardiovascular morbidity in ESRD [40]. The limitations of our study lie on the relatively small number of patients examined. Nonetheless, the results obtained are in line with the theory of chronic inflammation in dialytic patients and could stimulate larger prospective studies.

ACKNOWLEDGMENTS

K.K. was supported by the British Heart Foundation. The genetic research was conducted within the network of the London IDEAS Genetic Knowledge Park.

Reprint requests to Attilio Losito, M.D., via dei Mille 5 (San Marino), 06070 Corciano (PG), Italy.

E-mail: atlosito@tin.it

REFERENCES


