Endothelin-1 and F2-isoprostane relate to and predict renal dysfunction in hypertensive patients

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Abstract

Background. Hypertension and additional non-traditional risk factors can damage the kidney directly and by promoting atherogenesis. Evidence indicates that increased oxidative stress and inflammation may mediate a large part of the effects of risk factors on the kidney. We hypothesized that in hypertensive patients (HT), oxidative stress, measured as 8-ISO-prostaglandin F2alpha (8-ISO-PGF2alpha), should raise paralleling decreasing renal function and should correlate with estimated glomerular filtration rate (eGFR).

Methods. In 626 HT with renal function ranging from stages 1 to 5 and 100 healthy controls, plasma levels of 8-ISO-PGF2alpha, high-sensitivity C-reactive protein (CRP), transforming growth factor-beta (TGF-beta) and endothelin-1 (ET-1) were measured. GFR was estimated by the Modification of Diet in Renal Disease study equation.

Results. When HT were stratified according to renal function stages, 8-ISO-PGF2alpha, CRP, TGF-beta and ET-1 increased progressively and significantly with decreasing eGFR.

The multiple regression analysis, considering eGFR as a dependent variable, showed that 8-ISO-PGF2alpha ($\beta = -0.361$, $P < 0.000001$), ET-1 ($\beta = -0.197$, $P < 0.00001$) and TGF-beta ($\beta = -0.170$, $P < 0.0004$) correlated independently with eGFR. All biomarkers were good predictors of eGFR $< 60$ ml/min/1.73 m$^2$ [receiver-operator curve (ROC) areas]. ET-1 was shown to be the best predictor with a ROC area = 0.938; with a threshold of 4 pg/ml, 91% sensitivity and 85% specificity were observed, whereas 8-ISO had a ROC area = 0.931, and for a threshold of 329 pg/ml, sensitivity and specificity were 89%, respectively. In contrast, CRP showed the lower predictive value with a ROC area = 0.917; with a threshold of 2.52 mg/l, an 87% sensitivity and an 83% specificity were obtained.

Conclusions. Our findings are a clear-cut demonstration of a strong and negative correlation of both oxidative stress and ET-1 with renal function stages in HT. ET-1 and 8-isoprostane are predictive of eGFR.

Keywords: atherosclerosis; endothelium; inflammation; oxidative stress; renal dysfunction

Introduction

The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) has defined the stage of chronic kidney disease (CKD) based on the value of glomerular filtration rate (GFR) [1]. According to this classification, a GFR $\leq 59$ ml/min/1.73 m$^2$ defines a moderately decreased renal function.

Several studies have indicated the level of kidney dysfunction as an independent risk factor for cardiovascular outcomes both in communities and in high-risk populations [2,3].

Arterial hypertension is one of the main causes of kidney damage leading to end-stage renal disease (ESRD) [4,5], and cardiovascular disease is the leading cause of mortality in patients with renal disease [6].

Atherosclerosis integrates the response to a number of insults, and consequently, the accelerated atherosclerosis found in CKD patients is associated with the activation of a variety of humoral and tissue mechanisms.

The so-called response-to-injury hypothesis on atherosclerosis states that the initial damage involves the endothelium, leading to endothelial activation and dysfunction [7].

The concept that atherosclerosis is an inflammatory disease is well established [8].

There is considerable evidence that both endothelial changes and inflammation are associated with essential hypertension and with renal failure [9–12]. Moreover,
experimental evidence indicates that oxidative stress contributes to the pathogenesis of hypertension and may be involved in atherogenesis [13].

A common approach to estimate oxidative stress in vivo is to measure the end-products of lipid peroxidation. 8-ISO-prostaglandin F2alpha (8-ISO-PGF2alpha) is an index of lipid peroxidation endowed with vasoconstrictive and platelet-activating properties. Urinary excretion of 8-ISO-PGF2alpha is elevated in patients at risk for future cardiovascular events, and it is considered to be a useful index of oxidative stress [14–16]. Nitric oxide and endothelin-1 (ET-1) are reciprocally regulated [17], and an impaired availability of nitric oxide could lead to increased ET-1 production, inducing endothelial dysfunction. This latter is the triggering event in atherosclerosis and participates in maintaining vascular inflammation [17].

ET-1 elicits an inflammatory response by increasing oxidant stress in the vascular wall, which induces vascular remodeling and endothelial dysfunction [18,19].

It was shown that ET-1 exerts remarkable effects on renal haemodynamics consisting of decreased renal blood flow and GFR as well as increased renal vascular resistance [20–23].

We hypothesized that in hypertensive patients (HT), oxidative stress, measured as 8-ISO-PGF2alpha, should raise paralleling decreasing renal function, and should be inversely correlated with it. Further, the increase of oxidative stress should be associated with that of both inflammatory and pro-fibrotic molecules such as CRP, TGF-beta and ET-1.

Subjects and methods

In accordance with the Declaration of Helsinki and institutional guidelines, the protocol was approved by the local Ethical Committee and subjects were aware of the investigational nature of the study and agreed to participate after giving informed consent.

Study population

We considered 626 consecutive HT who were defined as hypertensives when clinic systolic/diastolic blood pressure (S/DBP) was >140/90 mmHg [24] or when treated with antihypertensive therapy.

HT were recruited among our outpatients attending our nephrology and hypertension unit for the differential diagnosis and/or treatment of their hypertensive disease. Study subjects underwent a detailed review of their medical history and routine laboratory measurements consisting of determination of serum and urinary creatinine and electrolytes, serum glucose, cholesterol, triglycerides, plasma catecholamines and renin activity, renal echography and a colour-Doppler of the main renal arteries.

Clinic BP was considered as the average of three consecutive measurements using a mercury sphygmomanometer after the subject had been sitting for 5 min.

Exclusion criteria were age <18 years and >70 years, known or evidenced diabetic disease, accelerated-malignant arterial hypertension, mineralocorticoid forms of hypertension, pheochromocytoma, history of transient ischaemic attack or stroke, history of coronary heart disease or myocardial infarction, heart failure, abnormalities of cardiac rhythm or conduction under pharmacological treatment, current or recent withdrawn treatment with statins.

All patients were hypertensives; among them 363 were not taking antihypertensive drugs (Table 1).

We show data of glomerular filtration rate estimated (eGFR) using the Modification of Diet in Renal Disease (MDRD) study prediction equation in all subjects [25].

On the day of the study, at 9 a.m., with the patients in a supine position and after fasting overnight, blood samples were obtained from an indwelling forearm venous catheter to assay 8-ISO-PGF2alpha, hs-CRP, TGF-beta and ET-1.

In the meantime, BP was measured.

Laboratory methods

All endothelium-derived parameters were measured by ELISA using a solid-phase specific sandwich
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Statistical analyses

Results are given as means ± SD.

According to the National Kidney Disease Education Program (NKDEP), there are some limitations to use of the MDRD study equation, and NKDEP recommends to report eGFR values > 60 ml/min/1.73 m² as > 60, and not as an exact number. For values < 60, the ‘report should give the numeric estimate rounded to the nearest whole number’ [26]. Therefore, we first analysed data from the 626 HT after dividing them into two groups, based on eGFR higher or lower than 60 ml/min/1.73 m².

Successively, patients having eGFR < 60 ml/min/1.73 m² were grouped according to the stage of their kidney function as indicated by K/DOQI [1]. Four groups were thus obtained: stages 1 and 2: eGFR > 60 ml/min/1.73 m² (n = 450); stage 3: eGFR 30–59 ml/min/1.73 m² (n = 182); stage 4: eGFR 15–29 ml/min/1.73 m² (n = 44) and stage 5: eGFR < 15 ml/min/1.73 m² (n = 36). The differences between the groups were evaluated using ANOVA and the Tukey post hoc test for multiple comparisons. Analysis of covariance (ANCOVA) was performed to adjust for potential confounders such as age, sex, glycaemia, BPs and previous treatment.

Simple and multiple regression analyses to test the relationships of estimated GFR with 8-ISO-PGF2alpha and other variables were used.

The multiple regression model was built considering eGFR as a dependent variable and including serum glucose, LDL, BMI, age, sex and anti-hypertensive treatment (coded as follows: 0: no treatment; 1: previous treatment; 2: ACEIs or ARBs; 3: diuretics; 4: beta-blockers; 5: central antihypertensives and 6: combination of two or more drugs).

The null hypothesis was rejected at a two-tailed P ≤ 0.05.

Results

Table 1 gives demographic data of healthy normotensive controls (NT) and HT.

The comparison between NT and HT demonstrated that in HT oxidative stress, measured as the plasma concentration of 8-ISO-PGF2alpha, was significantly increased. Plasma levels of ET-1, TGF-beta and hs-CRP were also augmented (Table 1).

Successively, the HT group was divided according to stages of kidney function [1] that is eGFR > 60 ml/min/1.73 m². Plasma levels of ET-1, TGF-beta and hs-CRP were also augmented (Table 1).

We further observed increasing levels of all other biomarkers along with the decreasing levels of eGFR, with significant differences between the groups in each parameter examined (Table 2).

A further analysis was carried out evaluating treated versus untreated patients. As expected, in the two groups significant differences in biomarkers were observed (Table 3). With ANCOVA, these differences were observed even after the adjustment for age, treatment and diastolic blood pressure, and in the absence of eGFR among covariates. In contrast, when eGFR was added as a covariate, all differences, including those between biomarkers, became
Table 2. Mean values of clinical and endothelial data divided according to stages of renal function

<table>
<thead>
<tr>
<th></th>
<th>Stages 1 and 2 eGFR &gt; 60 ml/min/1.73 m² (n = 450)</th>
<th>Stages 3–5 eGFR &lt; 60 ml/min/1.73 m² (n = 176)</th>
<th>eGFR &lt; 60 ml/min/1.73 m² (n = 450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-ISO PGF2alpha (pg/ml)</td>
<td>205 ± 90</td>
<td>404 ± 134*</td>
<td>323 ± 97</td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>3.27 ± 0.5</td>
<td>4.4 ± 0.94*</td>
<td>3.9 ± 0.47</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>1.75 ± 0.9</td>
<td>3.37 ± 1.37*</td>
<td>2.5 ± 0.76</td>
</tr>
<tr>
<td>TGF-beta (ng/ml)</td>
<td>27 ± 5.2</td>
<td>38 ± 8.8*</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>144.4 ± 19</td>
<td>139 ± 21**</td>
<td>141 ± 21</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>89 ± 13</td>
<td>80 ± 12</td>
<td>82 ± 13</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>84.86 ± 28.3</td>
<td>238.7 ± 168*</td>
<td>256.4 ± 61.91</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>87 ± 15</td>
<td>314 ± 15.8*</td>
<td>43.6 ± 9.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.5 ± 4.4</td>
<td>28 ± 5.7</td>
<td>28 ± 5.7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 12</td>
<td>62 ± 12*</td>
<td>62 ± 12</td>
</tr>
</tbody>
</table>

*P < 0.0001. Stages 3–5 versus stages 1 and 2.
**P < 0.002. Stages 3–5 versus stages 1 and 2.
†P < 0.0001. Stage 3 versus stage 4.
‡P < 0.0001. Stage 4 versus stage 5.
§P = NS after adjustment for eGFR (ANCOVA).


Table 3. Clinical and endothelial data of treated and untreated hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Treated (n = 263)</th>
<th>Untreated (n = 363)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 13</td>
<td>48 ± 13</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.3 ± 5.12</td>
<td>28.5 ± 4.52</td>
<td>0.039</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143.8 ± 21.2</td>
<td>143.5 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.4 ± 12.6</td>
<td>88.1 ± 13.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.1 ± 1.22</td>
<td>5.08 ± 1.06</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.71 ± 1.05</td>
<td>1.37 ± 0.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.17 ± 0.3</td>
<td>1.19 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>5.94 ± 1.86</td>
<td>5.66 ± 1.61</td>
<td>0.045</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>163.5 ± 142</td>
<td>108.7 ± 97.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>61.8 ± 29.2</td>
<td>82.3 ± 22.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>2.52 ± 1.36</td>
<td>2.24 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>3.86 ± 0.93</td>
<td>3.44 ± 0.74</td>
<td>0.0001*</td>
</tr>
<tr>
<td>TGF-beta (ng/ml)</td>
<td>33.2 ± 9.1</td>
<td>28.5 ± 6.95</td>
<td>0.0001*</td>
</tr>
<tr>
<td>8-ISO-PGF2alpha (pg/ml)</td>
<td>307 ± 153</td>
<td>234.3 ± 120</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*P = NS after adjustment for eGFR (ANCOVA).

HDL: high density lipoprotein; eGFR: MDRD estimated glomerular filtration rate; hs-CRP: high sensitivity C-reactive protein; ET-1: endothelin-1; TGF-beta: transforming growth factor-beta; 8-ISO-PGF2alpha: 8-ISO-prostaglandin F2alpha; NS: not significant.

Univariate and multivariate analyses of correlations of eGFR

In the 626 HT patients, the linear analysis of correlation indicated significant and inverse correlations of eGFR with 8-ISO-PGF2alpha (r = −0.713, P < 0.000001) (Figure 1), ET-1 (r = −0.686, P < 0.000001) (Figure 2), CRP (r = −0.172, P < 0.000015) and TGF-beta (r = −0.698, P < 0.0001).

A significant correlation of eGFR with DBP (r = 0.277, P < 0.00001) was observed.

The multiple regression analysis carried out considering eGFR as a dependent variable, and including 8-ISO-PGF2alpha, CRP, TGF-beta, ET-1, BP, serum glucose and LDL, age, BMI, sex and anti-hypertensive treatment, showed that in HT plasma levels of 8-ISO
ISO-PGF2alpha (0.897), TGF-beta (0.89) and CRP (0.86),
dependently of BP levels. Furthermore, the newest data aris-
ing progressively as renal function declines, indepen-
dent of oxidative stress, versus metabolism and excretion [34].

Isoprostanes can be produced locally in the kidney. It has
been shown that nanomolar administration of isoprostanes
into the rat produces a potent renal vasoconstriction of the
mechanism appears to be involved in sequential steps
of atherosclerosis, from endothelial dysfunction to plaque
formation and rupture. ROS can impair endothelial func-
tion and increase systemic and intrarenal proinflammatory
and fibrogenic factors, probably, triggering a sequence of
mechanisms involved in atherosclerosis and renal injury.

The results of this study confirm our hypothesis, demon-
strating that in hypertensive patients with renal function
ranging from normal to severe kidney failure stages [1],
plasma concentrations of the biomarker of oxidative stress
increase progressively as renal function declines, indepen-
dently of BP levels. Furthermore, the newest data aris-
ing from this study are the predictive power of 8-ISO-
PGF2alpha of declined eGFR. Indeed, this latter point
seems to confirm the relationship between atherosclerosis
and oxidative stress, as we demonstrated recently in patients
with coronary stenosis [28].

Renal function abnormalities may exist at the early stages
of atherogenesis. Several studies [29–31] have consistently
demonstrated the role of oxidative stress in experimental
and clinical renal injury.

The mechanism appears to be involved in sequential steps
of atherosclerosis, from endothelial dysfunction to plaque
formation and rupture. ROS can impair endothelial func-
tion and increase systemic and intrarenal proinflammatory
and fibrogenic factors, probably, triggering a sequence of
mechanisms involved in atherosclerosis and renal injury.

Isoprostanes are prostaglandin-like compounds formed
from the peroxidation of arachidonic acid, a ubiquitous
polyunsaturated fatty acid [32]. The sources of free radicals
that contribute to isoprostanes formation in vivo are mul-
tiple. These include the generation and leakage of reactive
oxygen species such as superoxide from the mitochondrial
electron transport system and the generation of superoxide
by the NADPH oxidases, among others [33].

An important point regarding the quantification of 8-
ISO-PGF2alpha in biological fluids is that their levels in a
certain tissue likely represent a steady-state concentration
that is dependent on production, thus degree of oxidative
stress, versus metabolism and excretion [34].

Roberts and Morrow [35,36] demonstrated that circulating
F2-isoprostane concentrations are dependent on produc-
tion rather than metabolism and excretion, suggesting
that they truly are indicative of the level of oxidative stress
in vivo. Consequently, it is likely that in the present study, the
increase in the oxidative stress biomarker paralleling less-
ening renal function is not merely due to altered metabolism
or clearance of 8-ISO-PGF2alpha but to an excess in ox-
diabetic patients [34].
afferent arteriole, which reduces GFR and renal blood flow [32,33,35].

In the present study, we further demonstrate by multiple regression analysis, considering eGFR as a dependent variable, that in hypertensive patients eGFR correlates inversely with 8-ISO-PGF2a. This was true even considering GFR estimated by the Mayo Clinic equation. Recently, a similar observation was reported in a very small group of patients with stage 1–4 CKD [37].

All these data are in contrast with that reported by Oberg et al. [38], who did not find any correlation of eGFR with neither plasma concentrations of thiol groups nor of the protein carbonyl group content and F2-isoprostanes in patients with moderate-to-severe CKD. These discrepant results may be due to causes such as the differences in the number of subjects, who were 60 patients in the report by Oberg et al. Further, plasma protein thiol oxidation and plasma protein carbonyl content undergo tubular metabolism rather than glomerular filtration to be cleared by the kidney.

Other major findings of this study are those regarding ET-1. Indeed, in hypertensives ET-1 plasma concentrations increase with decreasing eGFR, are negatively correlated with eGFR independently of BP levels and predict declining eGFR. We demonstrated previously that in chronic renal failure increased ET-1 plasma concentrations are not due mainly to a reduced clearance of the peptide [10]. It is in our opinion that the present progressive increases in ET-1 concentrations which parallel decreasing eGFR are not merely secondary to its reduced clearance.

It was shown that ET-1 exerts remarkable effects on renal haemodynamics [20–23]. Moreover, endothelin transgenic mice develop glomerulosclerosis [39]. Thus, the paracrine/autocrine renal endothelin system is suggested to be involved in the regulation of renal function. Attention has been paid to the possibility that ET-induced vasoconstriction may be dependent, at least in part, on the production of superoxide anion [23,29,40].

On the other hand, Hirai et al. [41] studied ET-1 in plasma in 1492 subjects and showed that normal creatinine concentrations were significantly correlated with plasma ET-1. No relation was demonstrated between plasma ET-1 and BP, suggesting that high ET-1 is not related to hypertension, but to subclinical renal dysfunction.

Our results seem to be in line with these data, in particular, considering the high predictive power demonstrated by ET-1 with regard to GFR < 60 ml/min/1.73 m². Nevertheless, it is to be considered that animal models of chronic renal failure show not only an increase in ET-1 production, but also a likely reduction in its clearance [42].

The two leading causes of ESRD are diabetes and hypertension [43], and even mild-to-moderate hypertension is a risk factor for the progression of CKD towards ESRD [4].

In our study, in spite of medications, treated patients were characterized by higher plasma values of biomarkers of endothelial dysfunction. This was not surprising, considering that these individuals were older and had a worse renal function than untreated patients. Indeed, when eGFR was considered as a covariate in the ANCOVA analysis, all differences between the two groups disappeared, indicating that renal function was the determinant of the differences between treated and untreated patients.

Our study demonstrates that in HT oxidative stress increases even in the early stage of eGFR decline, correlates to it and predicts its reduction.

To the best of our knowledge, this is the first study reporting data of the oxidative stress biomarker in relation to the five stages of renal function in a wide group of hypertensives. A possible weakness in our results could be represented by the influence of pharmacologic treatment, even if it was taken into account in the multivariate analysis.

A further limitation of our study is its cross-sectional, observational feature. Even if we demonstrate in a wide group of hypertensive patients the association of increased oxidant stress with renal function degree, we cannot state that enhanced oxidative stress leads to decreasing renal function.

In summary, our findings are useful in their clear-cut demonstration of a strong and negative correlation of increasing oxidative stress with decreasing renal function in hypertensive patients. Furthermore, ET-1 and 8-isoprostane are predictive of eGFR. This gives support for longitudinal studies aimed at evaluating the relationship between atherosclerotic complications and renal failure in a hypertensive population.

Acknowledgements. This work was supported in part by a grant from the Italian Ministry for University and Scientific Research (MURST) and by a Research grant from the Italian Society of Hypertension (SIIA).

Conflict of interest statement. None declared.

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Received for publication: 4.3.08 Accepted in revised form: 6.8.08