**Blood 8-hydroxy-2′-deoxyguanosine is associated with erythropoietin resistance in haemodialysis patients**

Akihiko Kato¹, Mari Odamaki² and Akira Hishida³

¹Division of Nephrology, Endocrinology and Metabolism, Shizuoka Cancer Center Hospital, Shizuoka, ²Department of Clinical Nutrition, School of Food and Nutritional Science, University of Shizuoka, Shizuoka and ³First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

**Abstract**

**Background.** 8-Hydroxy-2′-deoxyguanosine (8-OHdG), a product of oxidized DNA, is increased in haemodialysis (HD) patients, but the clinical relevance of enhanced 8-OHdG production in these patients remains unknown.

**Methods.** We cross-sectionally measured serum 8-OHdG in 73 patients on maintenance HD (age 68 ± 2 years, time on HD 85 ± 11 months, male/female = 42/31), and examined the relationship between blood 8-OHdG and the severity of renal anaemia and the weekly dosage of recombinant human erythropoietin (rHuEPO).

**Results.** There was a significant increase in serum 8-OHdG in HD patients compared with normal subjects. Serum 8-OHdG was positively correlated with the patients' age (r = 0.231, P < 0.05) but not with the duration of HD. Serum 8-OHdG was significantly higher in diabetic subjects than in non-diabetic subjects (P < 0.05). Serum 8-OHdG had a significant inverse correlation with haemoglobin (Hb) (r = -0.526, P < 0.01) but a positive correlation with the rHuEPO dose (r = 0.443, P < 0.01) and the ratio of the weekly rHuEPO dose divided by Hb (r = 0.487, P < 0.01). Serum 8-OHdG was not correlated with inflammatory and nutritional parameters.

**Conclusions.** These findings suggest that the elevation of circulating 8-OHdG may be associated, at least in part, with rHuEPO resistance in HD patients.

**Keywords:** anaemia; erythropoietin; haemodialysis; MDA; 8-OHdG

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**Introduction**

Recent studies have demonstrated that patients on chronic haemodialysis (HD) are more exposed to oxidative stress than normal subjects [1]. A higher circulating level of malondialdehyde (MDA) is noted in HD patients, suggesting accelerated lipid peroxidation as a consequence of multiple pathogenic factors [1].

Several studies show a possible association between enhanced oxidative stress and renal anaemia in dialysis patients [2–5]. For example, higher MDA levels in plasma and red blood cells (RBC) were negatively correlated with haemoglobin (Hb) levels [2]. Correction of anaemia by treatment with recombinant human erythropoietin (rHuEPO) greatly reduced plasma MDA levels [3]. Patients with lower plasma glutathione, a water-soluble antioxidant, were more susceptible to haemolysis induced by chloramine exposure [4]. In addition, increased MDA content was found in RBC membranes from HD patients with rHuEPO hyporesponsiveness [5], suggesting that oxidative stress itself may be a factor of resistance to rHuEPO.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant oxidative products of DNA [6]. Increased 8-OHdG content in urine and lymphocytes has been observed in a variety of diseases. A marked elevation of 8-OHdG in leukocyte DNA samples obtained from patients with chronic renal failure was recently reported [7–9]. In non-dialysed patients, 8-OHdG content in peripheral leukocytes gradually increased with the progression of renal failure [9]. A significantly higher level of 8-OHdG in leukocyte DNA was found in HD patients using cellulose membrane than in those using synthetic membrane [7,8].

Recently, an elevation of blood 8-OHdG was observed in neurodegenerative diseases [10]. A reduction in serum 8-OHdG by cholesterol-lowering therapy was also noted in dyslipidaemic subjects [11]. Satoh et al. [12] first showed high levels of serum 8-OHdG in subjects on maintenance HD. They found that blood 8-OHdG was increased by 133% of its pre-dialysis level after a single HD using a synthetic dialyser membrane [10]. In addition, vitamin E-coated cellulose dialyser membranes prevented this rapid increment of blood.

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**Correspondence and offprint requests to:** Akihiko Kato, MD, Division of Nephrology, Endocrinology and Metabolism, Shizuoka Cancer Center Hospital, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan. Email: a.kato@scchr.jp

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8-OHdG. Long-term use of this membrane, for 6 months, also gradually decreased blood 8-OHdG and other oxidative markers, such as MDA and advanced glycation end products [10]. These findings suggest that circulating 8-OHdG may be acutely produced by HD and may reflect the severity of systemic oxidative stress in patients on dialysis. However, there has been no study to examine the role of increased blood 8-OHdG in these patients.

The main goal of this study is to clarify the clinical relevance of blood 8-OHdG in HD patients. We cross-sectionally measured serum 8-OHdG, and examined the association between 8-OHdG and the degree of renal anaemia and rHuEPO responsiveness.

**Subjects and methods**

We selected 73 stable HD patients out of 384 at two dialysis units (Maruyama Hospital and Maruyama Clinic, Hamamatsu, Japan). We excluded from the study those patients who had acute infections, malignancy, cirrhosis and congestive heart failure. All patients were stable when assessed. The patients comprised 42 men and 31 women with an average age of 68 ± 2 (22–95) years. Their mean time on HD was 85 ± 11 months, ranging from 2 to 354 months. The causes of end-stage renal failure were primary renal anaemia and rHuEPO responsiveness, as this ratio yields a continuously

...distributed variable.

**rHuEPO responsiveness, as this ratio yields a continuously...**

subgroups according to their rHuEPO dosage: low (L), moderate (M), high (H), >75; and compared clinical parameters between the three groups. We also calculated the ratio of the weekly rHuEPO dose to Hct (rHuEPO/Hct) as another marker of rHuEPO responsiveness, as this ratio yields a continuously distributed variable.

**HD-related factors**

All patients had been undergoing regular HD for 4-4.5 h three times per week at a blood flow rate of 160–220 ml/min. All patients used bicarbonate dialysate (30 meq/l, Kindaly AF-2P, Fuso, Osaka, Japan) at a dialysis flow rate of 500 ml/min. All treatments were performed using one of the following membranes: low-flux ultrafiltration rate (UFR <20 ml/min h) modified regenerated cellulose hollow-fibre (MRC: AM-SD, Asahi Medical, Tokyo, Japan, or CL-EE, Terumo, Tokyo, Japan, n=9); medium-flux (UFR 20–40 ml/min h) cellulose triacetate hollow-fibre (CTA: FB-U, Nipro Medical, Osaka, Japan or TFW, Teijin-Gambro, Tokyo, Japan, n=39); and high-flux (UFR >40 ml/min h) polysulfone synthetic hollow-fibre (PS: BS-U, Toray Medical, Tokyo, Japan or APA, Asahi Medical, Tokyo, Japan, n=25). No patient in our group re-used a dialyser membrane. Blood samples were drawn from the arterial side of the arteriovenous fistula at the start and at the end of dialysis session after a 2-day interval from the last HD session. The efficiency of dialysis was assessed based on the urea reduction rate (URR)—calculated from monthly blood tests by the formula (1 — post-BUN/pre-BUN) × 100, and the delivered dose of dialysate (Kt/Vu) using a single-pool urea kinetic model. Protein catabolic rate (PCR), an indirect indicator of protein intake, was calculated from dialysis urea removal and serum urea levels.

**Analytical procedures**

Blood urea nitrogen (BUN), creatinine, total protein, albumin, total cholesterol, triglyceride, electrolytes and blood cell counts were measured by standard laboratory techniques using automatic analysers. C-reactive protein (CRP) was measured by laser nephelometer. Intact parathyroid hormone (PTH) was determined using an immunoradiometric assay. Serum ferritin was determined by the latex agglutination method. Blood-soluble tumour necrosis factor-α (TNF-α) receptor p80 (sTNFR p80), a sensitive marker of the activation of the systemic TNF-α system, was measured by a commercial ELISA kit [sTNFR (80 kDa) ELISA, Bender MedSystems, Vienna, Australia]. Serum 8-OHdG was measured using a commercially available competitive ELISA kit (Japan Institute for Control of Aging, Shizuoka, Japan) by diluting the samples. The kit can measure 8-OHdG values ranging from 0.125 to 10 ng/ml using a monoclonal specific antibody, N45.1 [13]. This antibody does not cross-react with the original four deoxyribonucleosides, 2'-deoxyinosine, 8-hydroxy-2'-deoxyadenosine or O2'-methyl-2'-deoxyguanosine.

**Statistical analysis**

Each value was expressed as the mean ± SE. Differences between two groups, patient and control, were analysed by an unpaired Student’s t-test following the ANOVA method. P values <0.05 were considered statistically significant. All statistical calculations were performed with GB-STAT software (Dynamic Microsystems, Silver Spring, MD).

**Results**

**Serum 8-OHdG and patient profile**

There was a significant increase in serum 8-OHdG in HD patients (21.31 ± 1.13 ng/ml, n = 73) compared with age-matched normal subjects (2.67 ± 0.41 ng/ml, n = 9). Blood 8-OHdG was significantly and positively correlated with age (r = 0.231, P < 0.05) but not HD duration (Figure 1). Diabetic patients (n = 20) also had a significantly higher 8-OHdG compared with non-diabetic patients (n = 53) (24.99 ± 2.69 vs. 19.93 ± 1.15 ng/ml, P < 0.05). In contrast, gender and sero-...
rHuEPO dose and clinical parameters

The rHuEPO dosage ranged from 0 to 236 U/kg/week at the assessment. The patients receiving >75 U/kg/week of rHuEPO (group H) were significantly older than those receiving rHuEPO <25 U/kg/week (group L) (P<0.05, Table 1). The prevalence of males was also significantly lower in group H than in group L (P<0.05). In contrast, there was no difference in HD duration and the prevalence of diabetes, HCV infection and iron supplementation between the three groups (Table 1).

Hb was significantly lower in group H compared with groups M and L (P<0.01, Table 1). The ratio of rHuEPO Hb was significantly higher in group H than in groups M and L (P<0.01, data not shown). Serum iron and total iron binding capacity (TIBC) also were significantly lower in group H than in group L (Table 1). Serum ferritin levels were identical between group H and L.

Serum creatinine was significantly reduced in group H compared with groups M (P<0.05) and L (P<0.01), respectively. The dosage of rHuEPO was significantly and inversely correlated with serum creatinine (r = −0.370, P<0.01) and albumin (r = −0.238, P<0.05). However, there were no differences in albumin, total cholesterol, triglyceride and PCR between the three groups (Table 1). No difference was found also in HD efficacy, such as Kt/Vurea and URR between any of the groups.

The serum 8-OHdG level was significantly lower in patients using the PS membranes (17.46 ± 1.39 ng/ml, n = 25) compared with those using the MRC (23.39 ± 2.23 ng/ml, n = 9) and CTA (23.31 ± 1.78 ng/ml, n = 39) (P<0.05) membranes. The usage of the PS membrane, however, was identical among the three groups.

rHuEPO dosage and serum 8-OHdG

Serum 8-OHdG was significantly higher in group H compared with groups M and L (P<0.01, Table 1). A significant and inverse relationship was found between Hb and 8-OHdG levels (r = −0.526, P<0.01) (Figure 2). Serum 8-OHdG was also significantly and positively correlated with rHuEPO dosage (r = 0.443, P<0.01). However, the correlation between serum 8-OHdG and rHuEPO dosage was not significant (r = 0.231, P<0.05) (Figure 1).

Dialysis-related factors

The patients receiving Hb supplementation were significantly older than those receiving iron supplementation (P<0.05). There were no differences in age, HD duration, HD-related factors, nutrition-related factors and inflammatory and oxidative markers between the three groups.

Table 1. Clinical parameters in HD patients receiving different rHuEPO dosage.

<table>
<thead>
<tr>
<th>Group (rHuEPO dosage)</th>
<th>L (&lt;25)</th>
<th>M (25≤ rHuEPO &lt;75)</th>
<th>H (≥75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>24</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Male/female</td>
<td>17/7</td>
<td>17/10</td>
<td>8/14</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Positive for anti-HCV antibody (%)</td>
<td>25</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Iron infusion (%)</td>
<td>13</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>rHuEPO-related factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rHuEPO (U/kg/week)</td>
<td>7 ± 2</td>
<td>46 ± 3b</td>
<td>148 ± 9b,c</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.2 ± 0.3</td>
<td>10.0 ± 1b</td>
<td>9.9 ± 0.2b</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>32.9 ± 0.9</td>
<td>29.5 ± 0.3b</td>
<td>28.8 ± 0.7b</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>206 ± 56</td>
<td>360 ± 81</td>
<td>384 ± 86</td>
</tr>
<tr>
<td>Fe (μg/dl)</td>
<td>71 ± 5</td>
<td>63 ± 4</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>TIBC (mg/dl)</td>
<td>239 ± 10</td>
<td>209 ± 5b</td>
<td>190 ± 7b</td>
</tr>
<tr>
<td>Nutrition-related factors</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8 ± 0.05</td>
<td>3.8 ± 0.05</td>
<td>3.7 ± 0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>167 ± 24</td>
<td>122 ± 13</td>
<td>110 ± 11</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>156 ± 9</td>
<td>151 ± 5</td>
<td>159 ± 8</td>
</tr>
<tr>
<td>PCR (g/kg/day)</td>
<td>0.96 ± 0.04</td>
<td>1.00 ± 0.03</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>Dialysis-related factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>69.1 ± 3.2</td>
<td>71.4 ± 2.5</td>
<td>68.6 ± 3.7</td>
</tr>
<tr>
<td>Crt (mg/dl)</td>
<td>11.6 ± 0.7</td>
<td>10.4 ± 0.4</td>
<td>9.1 ± 0.5bc</td>
</tr>
<tr>
<td>Kt/Vurea</td>
<td>1.23 ± 0.04</td>
<td>1.27 ± 0.04</td>
<td>1.36 ± 0.04</td>
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<td>URR (%)</td>
<td>70.2 ± 12</td>
<td>71.4 ± 1.2</td>
<td>73.9 ± 1.1</td>
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<tr>
<td>Intact PTH (pg/ml)</td>
<td>303 ± 42</td>
<td>241 ± 67</td>
<td>222 ± 31</td>
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<tr>
<td>PS membrane (%)</td>
<td>32.3</td>
<td>33.3</td>
<td>22.7</td>
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<tr>
<td>Inflammatory and oxidative markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.35 ± 0.17</td>
<td>0.39 ± 0.13</td>
<td>0.68 ± 0.27</td>
</tr>
<tr>
<td>sTNFR p80 (ng/ml)</td>
<td>22.2 ± 2.5</td>
<td>26.6 ± 3.7</td>
<td>24.4 ± 1.7</td>
</tr>
<tr>
<td>8-OHdG (ng/ml)</td>
<td>15.8 ± 1.5</td>
<td>22.0 ± 1.5b</td>
<td>26.5 ± 2.4b</td>
</tr>
</tbody>
</table>

Fe, iron; BMI, body mass index; Crt, creatinine.

*aP<0.05, bP<0.01, as compared with group L. cP<0.01, as compared with group M. Data represent the mean±SE.
P < 0.01) and rHuEPO/Hb ratio (r = 0.487, P < 0.01) (Figure 3). Serum 8-OHdG was not correlated with iron, TIBC or ferritin. In addition, serum 8-OHdG was identical between rHuEPO-treated patients with or without iron supplementation (17.04 ± 2.17 vs 22.16 ± 1.27 ng/ml, P = NS).

8-OHdG and inflammatory markers

Blood CRP in this study ranged from 0.0 to 5.8 mg/dl with a median of 0.1 mg/dl. Twenty-one patients (28.8%) exceeded the normal range (0.5 mg/dl). As the distribution of CRP was obviously not normal, we normalized the data by log transformation. Log CRP correlated inversely with Hb (r = −0.376, P < 0.05) and positively with rHuEPO dosage (r = 0.301, P = 0.06). In contrast, serum sTNFR p80 did not correlate with rHuEPO dosage and Hb. Nor did blood 8-OHdG correlate with CRP and sTNFR p80 level.

Discussion

In this study, we confirmed that blood 8-OHdG in HD patients was significantly higher than in general subjects. In addition, blood 8-OHdG had a positive correlation with age and was more elevated in diabetic subjects. These findings suggest that circulating 8-OHdG might reflect oxidative stress in normal ageing, diabetes and uraemia. However, our study did not identify the main source of blood 8-OHdG. In Parkinson’s disease and multiple system atrophy, a greater increase was observed in 8-OHdG in the serum compared with the cerebrospinal fluid, suggesting that increased blood 8-OHdG appears to be derived from peripheral tissues [14]. In dialysis subjects, as the 8-OHdG content of leukocyte DNA increases shortly after the start of dialysis [9], elevated circulating 8-OHdG may originate from the peripheral blood cells activated by the dialysis procedure.

In this study, we found that blood 8-OHdG was associated negatively with Hb and positively with the rHuEPO dose. Several mechanisms of oxidative stress-induced aggravation of renal anaemia have been demonstrated. For instance, increased oxidative stress may directly injure the DNA of early erythroid cells, for elevation of 8-OHdG has been noted in the bone marrow of aged rats [15]. Increased free radicals may also reduce EPO bioactivity directly by destroying tryptophan residues [16]. Additionally, oxidative stress may damage erythrocyte membrane and cause haemolysis [4]. As the 8-OHdG in the bone marrow is increased by aging [15], further studies are needed to determine if increased blood 8-OHdG reflects rHuEPO-induced increased proliferation or insufficient erythropoiesis in the bone marrow.

Recently, inflammation and malnutrition have been demonstrated to increase oxidative stress in
HD patients [17–19]. Blood CRP was correlated positively with markers of oxidative stress, such as plasma MDA and esterified F2-isoprostanes, whereas negatively with the plasma antioxidant, z-tocopherol [17,18]. Steinvinkel et al. [20] also found an increase of the oxidative marker, plasmalogen, in erythrocytes obtained from malnourished HD patients. In this study, however, serum 8-OHdG did not correlate with inflammatory and nutritional parameters. Tarng et al. [7] also noted no association between blood albumin and leukocyte 8-OHdG in dialysis patients. These findings could indicate that the increase of 8-OHdG levels in dialysis subjects is independent of micro-inflammation and poor nutritional status. We also found no correlation between HD efficacy and blood 8-OHdG.

In this study, significantly lower iron and TIBC levels were observed in group H. Blood iron has been shown as a determinant of the 8-OHdG content of leukocyte DNA in dialysis patients [7–9]. However, there was no correlation between blood 8-OHdG and iron parameters in this study. In addition, the dialysis patients who needed higher rHuEPO doses were older, and less frequently dialysed with PS membranes. As these parameters may increase those patients’ blood levels of 8-OHdG, a larger controlled study is needed to confirm a role for blood 8-OHdG in poor rHuEPO responsiveness.

The 8-OHdG content of leukocyte DNA is demonstrated to be genetically determined in HD patients. Tarng et al. [21] demonstrated that gene polymorphism of hOGG1, which deactivates 8-OHdG glycosylate, can influence leukocyte DNA levels. They found that 8-OHdG levels were significantly higher, by ~2-fold, in patients with the 1245GG genotype (38.1%) compared with patients with the 1245CG (51.9%) or CC genotypes (10.0%) while being independent of clinical parameters such as age, HD duration, blood antioxidant levels and iron status. In this study, we found that serum 8-OHdG was greatly increased in some subjects. So, it is possible that gene polymorphism of 8-OHdG could have influenced circulating 8-OHdG in this study.

There are some important limitations to this study. First, we only assessed 8-OHdG levels in serum but not in tissue—such as leukocyte. To our knowledge, there has been no study to compare 8-OHdG levels in blood and in leukocyte DNA. However, a recent experimental study showed that measurement of 8-OHdG in the plasma rather than tissue of diabetic rats is a more useful biomarker of oxidative DNA damage [22]. Next, our 8-OHdG levels seem much higher—probably a consequence of the cross-reactivity of our samples. Plasma 8-OHdG levels in control subjects are reported to be 10–15 pg/ml measured by a liquid chromatography-electrochemical-switching method [23]. Serum 8-OHdG in healthy subjects is also reported to be between 0.8 and 38 ng/ml [11,14], suggesting a variation of blood 8-OHdG by cross-reaction. As it is imperative to analyse the control and treated groups in the same study to obtain reliable 8-OHdG data [24], other studies are not comparable with our study, and further studies are needed to ascertain its significance using another assay. Thirdly, we did not assess other oxidative markers and antioxidants. Finally, we did not explore another role of increased 8-OHdG with respect to cancer development. Long-term observation could indicate the possible role of increased DNA damage in malignant transformation and cancer formation in dialysis patients.

In summary, we found that serum 8-OHdG, an abundant oxidative product of cellular DNA, was correlated with the severity of renal anaemia and rHuEPO dose. In contrast, nutritional and inflammatory status did not correlate with blood 8-OHdG. These findings suggest the possibility that increased oxidative stress may be associated, at least in part, with rHuEPO hyporesponsiveness in HD patients.

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