

## ORIGINAL ARTICLE

# Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis

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**Abstract**

**Background** The involvement of oxidative stress in the pathogenesis of various skin disorders has been suggested for decades. However, few clinical studies have assessed oxidative stress in skin diseases. The easiest and least invasive method to assess oxidative stress in patients may be the measurement of oxidation products in urine.

**Objective** This study aims to assess oxidative stress in psoriasis and atopic dermatitis patients.

**Methods** Urine samples were collected from 29 psoriasis patients (25 males and 4 females), 21 atopic dermatitis patients (14 males and 7 females) and 20 healthy controls (16 males and 4 females). The severity and extent of psoriasis and atopic dermatitis was assessed by their area and severity index. We measured nitrate as a metabolite of nitric oxide, malondialdehyde as a major lipid oxidation product, and 8-hydroxydeoxyguanosine (8-OHdG) as a DNA oxidation marker.

**Results** Urinary nitrate and 8-OHdG levels, but not malondialdehyde, were significantly higher in psoriasis patients than those in healthy controls. On the contrary, only urinary nitrate level was significantly higher in atopic dermatitis patients than those in healthy controls. The severity and extent of both psoriasis and atopic dermatitis significantly correlated with urinary nitrate level and malondialdehyde level, but it did not correlate with urinary 8-OHdG level.

**Conclusions** Measurement of these three urinary oxidative products is non-invasive. Above all, measurement of urinary nitrate may be most useful in the clinical assessment of oxidative stress in both psoriasis and atopic dermatitis patients. There is a possibility that urinary 8-OHdG level may indicate the different pathogenesis between psoriasis and atopic dermatitis.

Received: 20 January 2009; Accepted 21 April 2009

**Keywords**

atopic dermatitis, biomarker, oxidative stress, psoriasis vulgaris, urine

**Conflicts of interest**

None declared.

**Introduction**

The involvement of oxidative stress in the pathogenesis of various skin disorders has been suggested for decades.<sup>1,2</sup> However, few clinical studies have assessed oxidative stress in skin diseases.

The onset of oxidative stress has been related to excess formation, or defective detoxification, of reactive oxygen species (ROS)/reactive nitrogen species (RNS). This can arise from either up- or down-regulation of redox-related pathways or by defects of antioxidants. Resulting oxidative injury has been characterized through the following: DNA, protein, or lipid oxidation and excess ROS/RNS formation. Although some studies have revealed oxidation products in biological samples including tissue and blood, one of the easiest and least invasive ways for humans is to measure oxidation products in urine. Nitrate is a metabolite of nitric oxide (NO), which is a primary RNS.<sup>3</sup> Malondialdehyde may be one of major products of the lipid oxidation,<sup>4</sup> and 8-hydroxydeoxyguanosine

(8-OHdG) is an accepted marker of DNA oxidation.<sup>5</sup> These oxidation products are excreted in urine and can be measured by specific methods. Psoriasis and atopic dermatitis are different in nature and pathogenetically unrelated. However, these disorders share some common features in the inflammatory pathways.<sup>6</sup> The inflammatory pathways include oxidative stress, and oxidative stress has been linked to both psoriasis and atopic dermatitis.<sup>7,8</sup> In this study, we measured nitrate, malondialdehyde and 8-OHdG in the urine of both psoriasis and atopic dermatitis patients.

**Patients and methods****Patients**

Single urine samples were obtained from 29 psoriasis patients (25 males and 4 females), 21 atopic dermatitis patients (14 males and 7 females) and 20 healthy controls (16 males and 4 females), who

were selected from the outpatient clinic of the Department of Dermatology, Kagawa University Hospital. The patients had been under treatments, including corticosteroid ointment for psoriasis and atopic dermatitis, and vitamin D3 analogues ointment for psoriasis. Patients were not treated with systemic drug therapy, such as cyclosporine, methotrexate or biologic tumour necrosis factor- $\alpha$  inhibitors. Patients with severe co-existent disease, heavy smoking and alcohol intake were excluded. Informed consent was obtained from all patients. Twenty-nine psoriasis patients (age range: 21–76 years; mean: 55 years) and 12 healthy controls (age range: 25–95 years; mean: 54 years) were enrolled in an age-matched case-control study. Fifteen atopic dermatitis patients (age range: 21–33 years; mean: 28 years) and 12 healthy controls (age range: 23–38 years; mean: 26 years) were enrolled in an age-matched case-control study. The other six atopic dermatitis patients were not enrolled due to their inapplicable age. Disease severity of psoriasis was assessed with the Psoriasis Area and Severity Index (PASI). Disease severity of atopic dermatitis was assessed with the Eczema Area and Severity Index (EASI). Collected urine samples were stored, without any additives, at  $-80^{\circ}\text{C}$  until analysis.

#### Measurement of urinary oxidative products

Following thawing and centrifugation, the supernatants were applied to measurements. Urinary nitrate was measured as a metabolite of NO by the Griess reaction.<sup>9</sup> The concentrations of 8-OHdG were determined using a competitive enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Ageing, Shizuoka, Japan), following the manufacturer's instruction. Malondialdehyde was measured by reaction with thiobarbituric acid (Northwest Life Science Specialties, LLC, WA, USA). The values were all expressed as a relative to urinary creatinine (Cr) concentration that was obtained by using a commercially available kit (Cayman Chemical, MI, USA).

#### Statistics

Data are presented as mean and standard error (SE). Differences between groups were examined for statistical significance using unpaired *t*-tests. A *P*-value less than 0.05 denoted the presence of a statistically significant difference. Statistical analysis of correlation was performed by using the Spearman rank test.

#### Results

The urinary nitrate level was  $0.46 \pm 0.04 \mu\text{mol/mg Cr}$  in healthy controls and  $0.69 \pm 0.10 \mu\text{mol/mg Cr}$  in psoriasis patients (Fig. 1a). The urinary 8-OHdG level was  $4.79 \pm 1.30 \text{ ng/mg Cr}$  in healthy controls and  $10.64 \pm 2.32 \text{ ng/mg Cr}$  in psoriasis patients (Fig. 1b). The urinary malondialdehyde level was  $2.32 \pm 0.26 \text{ mmol/mg Cr}$  in healthy controls and  $2.62 \pm 0.18 \text{ mmol/mg Cr}$  in psoriasis patients (Fig. 1c). Of these three markers, urinary nitrate and 8-OHdG levels, but not malondialdehyde, were significantly higher in psoriasis patients than those in healthy controls ( $P = 0.038$ ,  $P = 0.034$ ,  $P = 0.856$ , respectively). There was a significant correlation between the

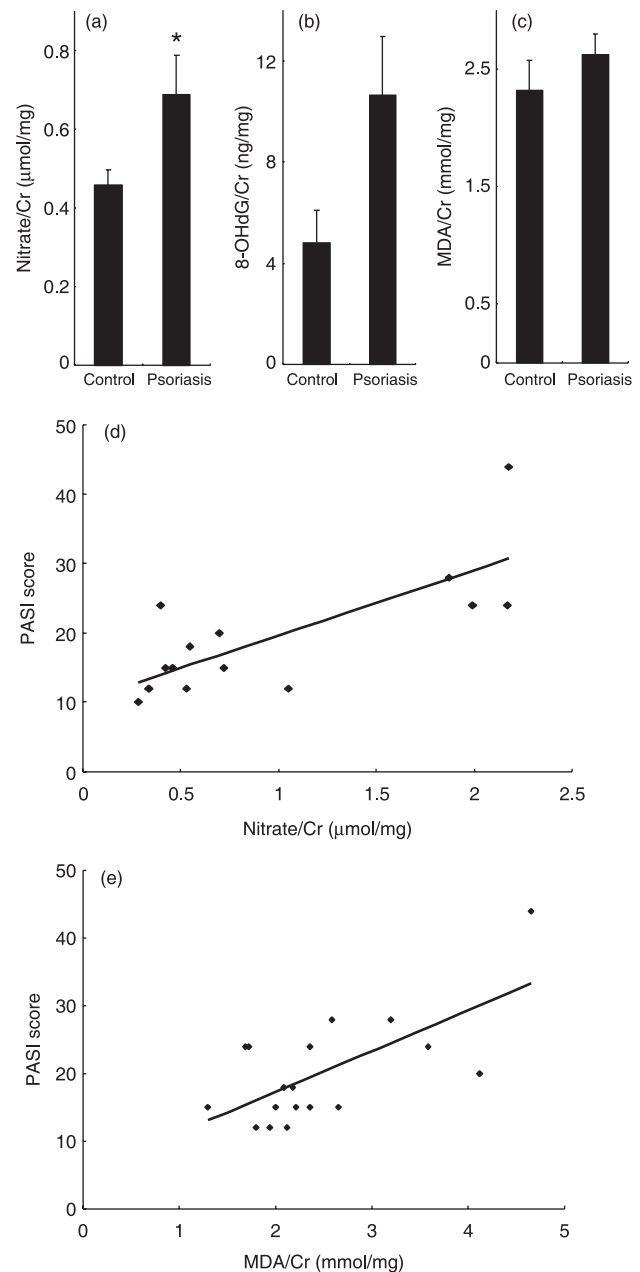


**Figure 1** Urinary oxidation products of psoriasis vulgaris patients. Age-matched case-control study; (a) urinary nitrate; (b) 8-OHdG; (c) malondialdehyde. Results are shown as mean  $\pm$  SE. \* $P < 0.05$  vs. healthy control group. Correlation with PASI score; (d) urinary nitrate level of patients with psoriasis reflected by PASI score  $> 9$ :  $r = 0.710$ ,  $P = 0.003$ ; (e) urinary malondialdehyde level of patients with psoriasis reflected by PASI score  $> 10$ :  $r = 0.488$ ,  $P = 0.040$ .

PASI score and urinary nitrate level (Fig. 1d;  $r = 0.710$ ,  $P = 0.003$ ), and urinary malondialdehyde level (Fig. 1e;  $r = 0.488$ ,  $P = 0.04$ ). We could not find a correlation between the PASI score and urinary 8-OHdG level.

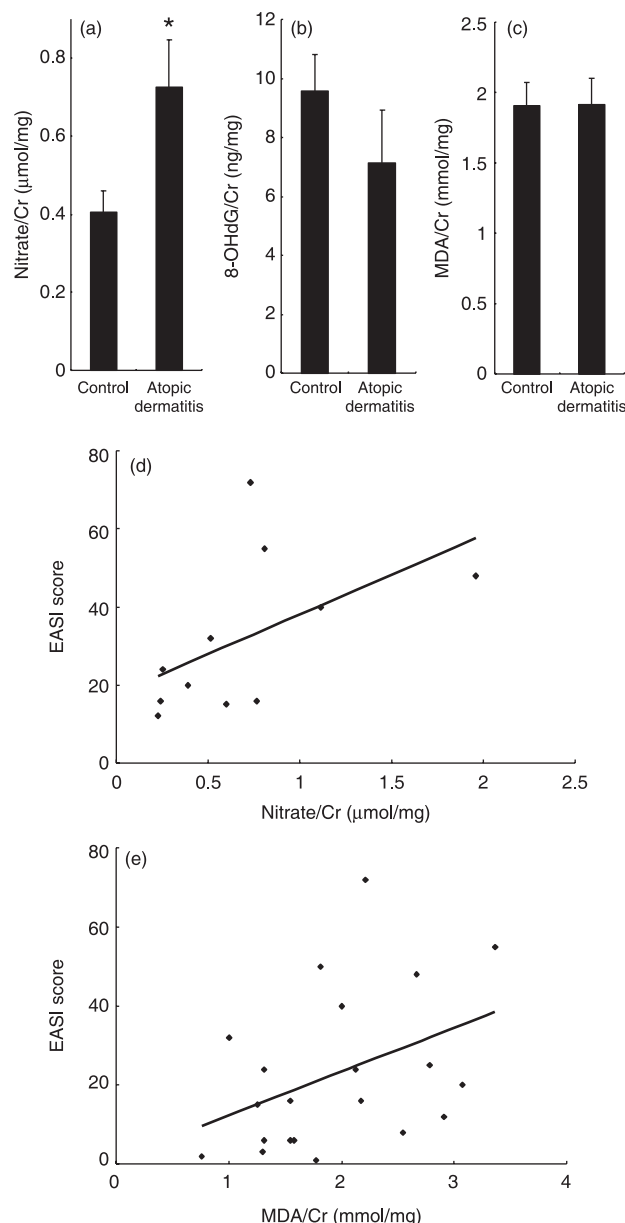


**Figure 2** Urinary oxidation products of atopic dermatitis patients. Age-matched case-control study; (a) urinary nitrate; (b) 8-OHdG; (c) malondialdehyde. Results are shown as mean  $\pm$  SE. \* $P < 0.05$  vs. healthy control group. Correlation with EASI score; (d) urinary nitrate level of patients with atopic dermatitis reflected by EASI score  $> 10$ :  $r = 0.647$ ,  $P = 0.0314$ ; (e) urinary malondialdehyde level of patients with atopic dermatitis reflected by EASI score  $> 1$ :  $r = 0.472$ ,  $P = 0.031$ .

The urinary nitrate level was  $0.40 \pm 0.06$   $\mu\text{mol/mg}$  Cr in healthy controls and  $0.72 \pm 0.12$   $\mu\text{mol/mg}$  Cr in atopic dermatitis patients (Fig. 2a). The urinary 8-OHdG level was  $9.59 \pm 1.23$  ng/mg Cr in healthy controls and  $7.13 \pm 1.80$  ng/mg Cr in atopic dermatitis

patients (Fig. 2b). The urinary malondialdehyde level was  $1.91 \pm 0.17$  mmol/mg Cr in healthy controls and  $1.91 \pm 0.19$  mmol/mg Cr in atopic dermatitis patients (Fig. 2c). Of these three markers, only urinary nitrate level, but not 8-OHdG or malondialdehyde, was significantly higher in atopic dermatitis patients than those in healthy controls ( $P = 0.026$ ,  $P = 0.271$ ,  $P = 0.972$ , respectively). There was a significant correlation between the EASI score and urinary nitrate level (Fig. 2d;  $r = 0.647$ ,  $P = 0.03$ ), and urinary malondialdehyde level (Fig. 2e;  $r = 0.472$ ,  $P = 0.03$ ). We could not find a correlation between the EASI score and urinary 8-OHdG level.

## Discussion

In this study, the urinary nitrate, 8-OHdG and malondialdehyde were studied to evaluate their use as biomarkers for oxidative damage in psoriasis and atopic dermatitis. Urine is considered an ideal biological material for the monitoring of oxidative stress because it can be obtained noninvasively.

A role for NO has been implicated in a variety of biological processes associated with increased circulating concentrations of cytokines and endotoxin. Although the involvement of cytokines and endotoxin is suggested in both psoriasis and atopic dermatitis, few clinical studies have assessed NO in these diseases. Determination of the NO itself is difficult due to its very short half-life. Therefore, measurement of the end products of NO, nitrate and nitrite, is most often used as a marker for the production of NO. Measurement of nitrate in blood may be direct assessment of the role of NO in diseases. Indeed, the elevation of blood nitrate levels and significant correlation with disease severity has been reported in psoriasis patients.<sup>10</sup> However, blood sampling may be disagreeable and invasive compared to urine sampling for patients. We have demonstrated for the first time the elevation of urinary nitrate levels and significant correlation with disease severity in psoriasis patients. We have also demonstrated the elevation of urinary nitrate levels and significant correlation with disease severity in atopic dermatitis patients. Despite the fact that the increased levels of NO products in serum<sup>11</sup> and bronchial exhalation<sup>12</sup> have been reported, previous reports about urinary nitrate levels in atopic dermatitis patients are controversial. Gutgesell *et al.* measured nitrate in daily urine sample, but they did not find a correlation with the atopic dermatitis skin status as assessed by the SCORAD.<sup>13</sup> Interestingly, low levels of single urinary nitrate have been reported in cases of childhood atopic dermatitis.<sup>14</sup> We are uncertain about these discrepancies at this stage. However, not only measurements in larger group of patients but also newly devised methods may be necessary to determine the role of NO in atopic dermatitis by taking urine sample.

Inflammatory pathways in psoriasis and atopic dermatitis include oxidative stress, and oxidative stress may cause DNA damage. In the case of oxidative damage to DNA, damaged products are usually eliminated by repair enzymes and detected as nucleoside derivatives. Urinary 8-OHdG is one adduct of this reaction and is proposed as a sensitive biomarker of oxidative

DNA damage and repair. 8-OHdG is a relatively abundant and readily detected product of oxidative DNA damage and as such is regarded as a useful and relevant marker for oxidative stress. We show here that the urinary 8-OHdG levels were significantly elevated in psoriasis patients (median age: 55 years) compared to controls. However, a previous study did not detect elevated levels of urinary 8-OHdG in psoriasis patients (median age: 36 years).<sup>15</sup> As 8-OHdG levels are elevated in ageing-related genetic diseases,<sup>16</sup> it is suggested that the DNA is easily oxidized in older psoriasis patients. Tsuboi *et al.* have demonstrated the elevated daily urinary 8-OHdG levels and the correlation between SCORAD index in atopic dermatitis patients.<sup>8</sup> Since taking single urine is easier than daily urine, we have tried to show the elevated single urinary 8-OHdG levels and the correlation between the disease severity of atopic dermatitis. However, single urinary 8-OHdG levels were not elevated and did not correlate with EASI score in atopic dermatitis patients.

A number of techniques have been developed for the measurement of lipid peroxidation products either as the lipid hydroperoxides themselves (primary products of lipid peroxidation) or as their degradation products (secondary products). The most widely used index of lipid peroxidation is malondialdehyde assay. Our study represents the first report of urinary malondialdehyde levels in psoriasis and atopic dermatitis patients. It is reported that blood malondialdehyde levels are elevated in psoriasis patients.<sup>17</sup> However, the urinary malondialdehyde levels were not elevated in psoriasis patients. There is no report measuring malondialdehyde levels in atopic dermatitis patients. Urinary acrolein-lysine levels, a similar lipid peroxidation product to malondialdehyde, are reported to be high in atopic dermatitis patients.<sup>18</sup> However, the urinary malondialdehyde levels were not elevated in our atopic dermatitis patients. Interestingly, the malondialdehyde level correlated with disease severity of both psoriasis and atopic dermatitis, implying that the measurement of urinary malondialdehyde levels could be utilized for measuring disease severity of psoriasis and atopic dermatitis.

## Conclusion

To our knowledge, this is the first report that represents urinary nitrate, 8-OHdG and malondialdehyde in patients with psoriasis and atopic dermatitis. Above all, measurement of urinary nitrate may be most useful in the clinical assessment of oxidative stress in both psoriasis and atopic dermatitis patients. There is a possibility that urinary 8-OHdG level may indicate the different pathogenesis between psoriasis and atopic dermatitis. The measurement of urinary malondialdehyde level may also merit further investigation. It still needs to be determined whether the identified biomarkers of

oxidative damage in psoriasis and atopic dermatitis would be applicable to other skin diseases. Therefore, further studies are required.

## Acknowledgements

This work was supported by grants-in-aid for scientific research to K. Nakai from the Shiseido Basic Research Foundation of the Japanese Dermatological Association. We thank Ms Fumiko Nishiyama for technical help and Mr Ian Willey for editorial help in the preparation of this manuscript.

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