

INVESTIGATIVE REPORT

Serum Antioxidant Capacity in Polymorphic Light Eruption

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Polymorphic light eruption is one of the few dermatological diseases in which some antioxidants have been said to be reduced in both the epidermis and the blood. This study measured the hydrosoluble antioxidant capacity in the serum of patients with polymorphic light eruption, using a commercially available kit. All patients were tested in winter, in order to avoid the influence of exposure to ultraviolet light. The results showed that a hydrosoluble antioxidant capacity was significantly decreased (by 29%) in patients with polymorphic light eruption, and b) that females (both patients and controls) has less hydrosoluble antioxidant capacity (by 27%) than males. In addition, the hydrosoluble antioxidant capacity values for females with polymorphic light eruption increased significantly with age, possibly accounting for the well-known propensity of young women to polymorphic light eruption. These last observations have not been reported previously. Key words: antioxidant; polymorphic light eruption; ageing.

(Accepted December 8, 2006.)

Acta Derm Venereol 2007; 87: 228–230.

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Free radicals and reactive oxygen species (ROS) are thought to be involved in ageing (1, 2) and some disorders, such as cancer, atherosclerosis, diabetes, viral and neurodegenerative diseases (1, 3, 4), either through their increased production or by the diminished levels of their inhibitors, the antioxidants. In fact, it has been suggested that antioxidants may delay or prevent both ageing and diseases, and their cutaneous application and oral administration have been suggested (5).

Polymorphic light eruption (PLE) is one of few dermatological diseases in which some antioxidants have been said to be reduced in both the epidermis and the blood (6, 7). To examine this further, we measured the hydrosoluble antioxidant capacity (HAOC) in the serum of patients with PLE. HAOC refers to uric acid, vitamin C, bilirubin, thiols and glutathione.

MATERIALS AND METHODS

Subjects

Twenty patients (10 men and 10 women) with PLE aged 45.1 ± 12.4 years (range 23–64 years) were studied. PLE clinical

diagnosis was supported by ultraviolet (UV) reproduction of lesions by photo-provocation test. Twenty-five healthy volunteers (17 men and 8 women) (mean age 40.6 ± 11.7 (range 19–68) years) without any dermatological disease entered the study as controls. Informed consent was obtained from all subjects.

All patients and subjects were studied in winter in order to avoid the influence of UV irradiation, and blood samples were taken after a 12 h fast to exclude the possible influence of consumption of beverages such as tea and coffee (8).

Laboratory analysis

HAOC was measured with a commercially available kit, PAO, supplied by MED.DIA (San Germano Vercellese, Italy). The HAOC Kit includes: uric acid as reference standard, buffer solution with bathocuproine disulphonate (BC), Cu^{++} solution and ethylenediamine-tetra-acetic acid (EDTA) stopping solution.

All samples were measured by Gralix Microplate Reader Spectra II (Gralix, Austria). The technique quantifies the antioxidant activity of the sample by measuring Cu^+ produced by the reduction of a known amount of Cu^{++} . Cu^+ is quantified using BC, which specifically binds Cu^+ . The Cu^+ -BC complex has a maximal absorbance at a wavelength of 480 nm, permitting the specific quantification of Cu^+ . The capacity of uric acid to reduce Cu^{++} is used to generate a standard curve by serial dilution of known amounts of uric acid. Samples are read at 480 nm before (first run) and 3 min after EDTA-stopping solution is added (second run). The values of absorbance obtained by subtracting the first reading from the second are matched to the standard curve, and the total antioxidant capacity is expressed in $\mu\text{mol/l}$ (9).

Sera were diluted 1:40 in Tris buffer solution containing BC, and the samples placed in a 96-well plate. For the blank, only the buffer solution was used. At a wavelength of 480 nm, 50 μl of a solution containing Cu^{++} was added and incubated at room temperature for 3 min. Fifty μl of EDTA stop solution was then added and a second run was performed at a wavelength of 480 nm.

Our data were obtained by triple analysis, within 1–2 weeks in order to avoid possible auto-degradation of the samples. In a separate experiment, we established that 14% auto-degradation occurred after 2–3 months (data not shown).

Statistical analysis

The data were analysed by Student's *t*-test and factorial analysis of variance (ANOVA). The correlation index (*r*) was calculated between age and HAOC values.

RESULTS

There was no significant difference in age between men and women ($t=1.179$; $p=0.245$) or between patients and controls ($t=1.249$; $p>0.219$) in this study. In addition, HAOC values did not correlate significantly with the

Table I. Hydrosoluble antioxidant capacity (HAOC) values in controls and patients with polymorphic light eruption (PLE)

HAOC	"Disease"		"Gender"		Males		Females	
	PLE	Controls	Males	Females	PLE	Controls	PLE	Controls
<i>n</i>	20	25	27	18	10	17	10	8
Mean \pm SD ($\mu\text{mol/l}$)	1006.1 \pm 223.2	1301.4 \pm 270.6	1311.8 \pm 267.3	957.6 \pm 164.4	1115.9 \pm 232.8	1427.1 \pm 218.0	896.3 \pm 155.5	1034.3 \pm 150.0

The difference between PLE and controls was highly significant ($F=230.792, p<0.001$).

The difference between males and females was highly significant ($F=34.226; p<0.001$).

The interaction "disease \times gender" was not significant. In other words, the diminution observed in males with PLE was not different from that observed in females.

SD: Standard deviation

age of the subjects, except in the group of women with PLE, in which HAOC values increased with age in a highly significant way ($r=0.897; p<0.001$). The other results are shown in Table I.

The HAOC mean value in patients with PLE (1006.1 \pm 223.2 $\mu\text{mol/l}$) was 29% lower than in controls ($F=230.8, p<0.001$). In females (both controls and cases), HAOC values were significantly lower (-27%) than in males (957.6 \pm 164.4 vs. 1311.8 \pm 267.3 $\mu\text{mol/l}$; $F=34.3; p<0.001$). There was no significant correlation between gender and disease, i.e. the reduction observed in males with PLE was not different from that observed in females.

DISCUSSION

Free radicals are molecules with unpaired electrons in their external orbitals. Such molecules tend to restore the external orbitals by exchanging electrons with other molecules, which, in turn, become free radicals, thus perpetuating the "radical cascade". Thus, free radicals are unstable and aggressive to biological tissues.

The skin, our outermost barrier, is directly exposed to environmental factors, such as electromagnetic radiation, smoke and chemical or biological pollution, which may all generate free radicals or ROS. As a protection system, the skin, however, has defence mechanisms, enzymatic as well as non-enzymatic (10), which block the progression of the oxidative sequence and repair the damaged intra- and extra-cellular structures.

Deficiencies of some antioxidants (catalase in the epidermis (6) and catalase, superoxide-dismuthase and vitamin E in the blood (7)) have been demonstrated in patients with PLE and solar urticaria (11). Our results, showing that patients with PLE have decreased HAOC values, are in keeping with such evidence.

One of our findings, which has not been reported previously, is that female subjects, whether controls or patients, had significantly lower HAOC values than male subjects.

It is well known that PLE occurs mainly in women, but the reason is not clear. Sex hormones may play a role (12) and, in one study, PLE and the use of contraceptive pills concurred in 15.3% of cases (13). In addition, 17 β -oestradiol prevents UV-induced suppression

of the contact hypersensitivity, a likely pathogenetic mechanism of PLE, induced by the release of immunosuppressive cytokines such as IL-10 (14). Another interesting finding of our study is the significant age-related increase in HAOC in females with PLE. While this result does not concur with previous, yet controversial (15, 16), data on the diminution of antioxidant capacity in elderly people (17–20), it may explain why PLE mainly affects young women.

We suggest, therefore, that the lower level of antioxidants may be the primary factor that accounts for a particular propensity of women to PLE. Larger studies are required to examine this further.

ACKNOWLEDGEMENT

We thank Maura Ratto, PhD, for her invaluable technical assistance.

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