

## Impact of oral vitamin E supplementation on oxidative stress & lipid peroxidation in patients with polymorphous light eruption

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Received March 16, 2005

**Background & objectives:** Polymorphous light eruption (PMLE) is a photo-induced disease which clinically manifests in the form of pruritic eruptions on sun/light exposed parts. Little is known about lipid peroxidation and free radical scavengers in patients during PMLE. The present study was therefore undertaken to evaluate oxidative stress and levels of antioxidant enzymes in patients of PMLE.

**Methods:** The PMLE was diagnosed clinically by a consultant dermatologist and validated independently by another and through histopathologic findings. Blood samples were collected on day 1 and patients were given oral vitamin E supplementation (400 mg OD) along with topical sunscreen and advice for photo-protection. Samples were collected again after one week. The blood samples were evaluated for lipid peroxidation, oxygen free radical (OFR) scavenging enzymes, glutathione (GSH) and related enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx), gamma glutamyl transpeptidase (GGT) and glutathione-S-transferase (GST) in erythrocytes and compared with healthy controls.

**Results:** The serum malondialdehyde (MDA) level was higher and GSH level was lower in PMLE cases as compared to controls. There was a significant decrease in superoxide dismutase (SOD) activity while activities of catalase (CAT) and glutathione related enzymes were increased in PMLE cases. Administration of oral vitamin E for one week, along with photoprotection resulted in a significant decrease in MDA levels and activities of all others enzymes except SOD. The GSH was replenished and returned to normal.

**Interpretation & conclusion:** Oxidative stress and differential modulation of antioxidant enzymes in PMLE might play a pathogenic role in humans, which supports the incorporation of antioxidant drugs in the treatment protocol of the disease.

**Key words** Antioxidants - free radical scavengers - polymorphous light eruption - sunburn - vitamin E

Although polymorphous light eruption (PMLE) is a common disease, the molecular mechanism(s) involved in pathogenesis are not clearly defined. The eruption of PMLE is induced by UV-irradiation<sup>1</sup>, bright summer sunlight usually being the most effective and is reportedly responsible for approximately 55 per cent of cases. Artificial induction has been successfully performed using ultraviolet (UV) A (narrow band) as well as UVB irradiation<sup>1</sup>. The pathogenic mechanism is thought to be a cell-mediated (delayed type) immune (hypersensitivity) response to UVR induced formation of neo-antigens in the skin<sup>2</sup>. Serial biopsies taken following UV exposure of susceptible patients have demonstrated T-cell infiltration of irradiated skin initiated by CD<sub>4</sub><sup>+</sup> (5 h) followed by CD<sub>8</sub><sup>+</sup> cells (72 h)<sup>3</sup>. Attempt for isolation of the inducing antigen in PMLE has not been successful, but apparent precipitation of the condition following allergic contact dermatitis to Fentichlor suggests an endogenous Fentichlor like antigen as one possibility<sup>4</sup>. Reactive oxygen species (ROS) are implicated in UV light induced damage to skin<sup>5</sup>. This oxidative damage could also be an initiator in the pathogenesis of skin cancer and photoageing<sup>6</sup>. It is known that UVA and UVB damage of skin may be accompanied by depletion of antioxidants<sup>7,8</sup>.

Oxygen free radicals (OFR) are highly reactive molecules and have the potential to damage various biomolecules *e.g.*, lipids, DNA, proteins, *etc.* Lipid peroxidation by OFR is frequently measured to assay the extent of OFR mediated damage<sup>9</sup>. Antioxidants that protect skin against OFR include various low molecular weight antioxidants such as ascorbate, glutathione (GSH), tocopherol and ubiquinol and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and thioredoxin reductase<sup>7,8,10</sup>.

In animals, systemic or topical application of vitamin C or E has been shown to exert a photoprotective action<sup>11,12</sup>. A number of studies

have emphasized the importance of administration of antioxidants such as ascorbic acid,  $\beta$ -carotene and/or  $\alpha$ -D-tocopherol for protection against UV-induced skin reaction<sup>13-15</sup>. Information is lacking on oxidative stress and lipid peroxidation in human cases with PMLE. The present study was thus designed to explore the presence of oxidative stress during PMLE in humans, how the different enzymes regulating oxidative stress are modulated during PMLE activity, and whether systemic administration of vitamin E can ameliorate these effects.

### Material & Methods

**Chemicals:** NADPH, oxidized and reduced glutathione, 1, chloro, 2,4, dinitrobenzene (CDNB), glutathione reductase and bovine serum albumin (Fraction V) were obtained from Sigma Chemical Company (St. Louis, Mo, USA). Pyrogallol and 2-thiobarbituric acid (TBA) were obtained from E. Merck (Mumbai, India). All other reagents used were of analytical grade and obtained either from BDH or SISCO Chemicals (Mumbai, India).

**Cases and controls:** The study group consisted of 20 patients, all male (age 22 to 44, mean 35 $\pm$ 5 yr), attending the outpatients clinic of the Department of Dermatology, Guru Teg Bahadur (GTB) Hospital, Delhi, India, during the year March 2002-May 2003. Patients clinically diagnosed to have PMLE by a dermatologist on the basis of the following criteria, were included in the study: (i) recurrent pruritic eruption clearly precipitated/aggravated by sun exposure; (ii) eruption predominantly confined to sun exposed area; (iii) monomorphic/uniform type of lesion(s) in each patient; (iv) history of relief over time following sun protection/sun screening in earlier episodes; and (v) skin biopsy consistent with PMLE.

Patients with active infection, eczema or history of any other photo aggravated dermatoses, or with any other known cause or modifier of oxidative stress parameters like diabetes, hypertension, *etc.*

or on drugs known to cause photodermatitis, eruptions/oxidative stress *e.g.*, tetracycline, quinolones, corticosteroids, were excluded.

Initially, 45 patients selected consecutively, were included in the study and some (n=8) were excluded on account of exclusion criteria. Seventeen patients did not complete the study or turn up for follow up assessment at 1 wk or came too late for inclusion. So, at the end of the study data from 20 patients only could be evaluated. However, the characteristics of these 20 patients were not different from those 25 who could not be included.

The study protocol was approved by Ethics Committee of the Institution. All patients had active disease/eruptions when their samples were first taken. All the patients were advised to use topical sunscreen, observe strict photoprotection and were given oral vitamin E supplementation (400 mg  $\alpha$ -tocopherol acetate OD) for 7 days. Twenty, age and sex matched healthy controls were selected from amongst students, hospital staff or their families. Written consent for the study was obtained from both patients and controls.

*Samples:* Blood (15 ml) was collected from each case on day one and after one week of treatment, and from control subjects once by venepuncture. All the patients had marked clinical improvement and/or remission by the time the second sample was collected. Ten ml of blood was collected in a tube containing heparin and another 5 ml was allowed to clot in a plain vial to separate serum. Hb concentration was determined spectrophotometrically at 540 nm using Drabkin's reagent. Erythrocytes were isolated and haemolyzed. Protein content of haemolysate was estimated by the method of Lowry *et al*<sup>16</sup>. Red cells were stored at 4°C and serum samples at -20°C.

*Lipid peroxidation:* The lipid peroxidation level in serum was measured as thiobarbituric acid reactive substances (TBARS) following the method described by Satoh<sup>17</sup>.

*Antioxidant enzymes:* SOD and catalase activities in erythrocytes were determined using standard methods<sup>18,19</sup>.

*Glutathione and related enzymes:* Total (GSH) content in blood was measured by the method of Tietze<sup>20</sup> using dithionitrobenzene and expressed as moles/ml. GR activity in plasma was determined by following the oxidation of NADPH to NADP during the reduction of oxidized glutathione and expressed as  $\mu$ moles of NADPH oxidized/min/ml<sup>21</sup>. Total activity of GPx in red cell haemolysate was determined<sup>22</sup>. Serum GST was measured spectrophotometrically by the method of Habig *et al*<sup>23</sup> using 1, chloro-2,4 dinitrobenzene as substrate. Gamma glutamyl transpeptidase (GGT) was measured by the method of Novogrodsky *et al*<sup>24</sup>.

*Statistical analysis:* The data were analysed by using SPSS PC (version 5.0) software. Paired t-test was employed for the comparison of data between the groups.

## Results

A 37 per cent increase in concentration of serum MDA was found in PMLE cases as compared to the control group ( $P < 0.001$ , Table I). SOD activity and blood GSH level were decreased by 12.5 and 30 per cent respectively while activities of CAT, GPx, GR, GST and GGT were increased in PMLE patients as compared to control (18, 19, 117, 54 and 94 per cent respectively, Tables I and II). Administration of oral vitamin E to the patients (400 mg OD) for 1 week along with sunscreen and photoprotection resulted in a significant decrease in MDA level and CAT activity, reaching near normal levels. However, there was no significant change in SOD activity (even after vitamin E treatment) and this parameter failed to reach normal levels (Table I). The GSH level was apparently replenished after vitamin E treatment and returned to normal. Similarly, GR, GST, GPx and GGT activities reached normal levels, similar to controls, after vitamin E supplementation (Table II).

**Table I.** Malondialdehyde level (in serum) and activity of antioxidant enzymes (in RBC) and glutathione level (in blood) of control subjects and PMLE cases

Parameter	Control subjects	PMLE cases	PMLE cases after vitamin E supplementation
Malondialdehyde (n.mol/ml)	2.30 ± 0.36	3.17 ± 0.26* (137)	2.42 ± 0.36** (105)
Superoxide dismutase (U/g Hb)	1652 ± 191.93	1447 ± 100.30* (87.5)	1480 ± 120.83* (89.5)
Catalase (U/g Hb)	2.67 ± 0.42	3.15 ± 0.39* (117.9)	2.58 ± 0.36** (96.6)
Glutathione (µmol/ml)	257.40 ± 24.14	179.60 ± 17.62* (69.7)	250.5 ± 14.24** (97.3)

Values are mean ± SD (n=20)

Figures in parentheses indicate per cent of control.

$P < 0.001$  compared to \*control and \*\*PMLE

PMLE, polymorphus light eruption

**Table II.** Activities of glutathione related antioxidant enzymes in blood of control subjects and PMLE cases

Parameter	Control subjects	PMLE cases	PMLE cases after vitamin E supplementation
Glutathione reductase (U/ml)	0.88 ± 0.14	1.91 ± 0.4* (217)	0.89 ± 0.13** (101)
Glutathione-S-transferase (n mole/mg protein)	0.87 ± 0.15	1.34 ± 0.17* (154)	0.81 ± 0.18** (93)
Glutathione peroxidase (U/g Hb)	5.76 ± 0.37	6.88 ± 0.34* (119)	5.67 ± 0.30** (98)
Gamma glutamyl transpeptidase (KA units/l)	32.90 ± 9.87	64.00 ± 14.70* (194)	28.50 ± 9.2** (86)

Values are mean ± SD (n=20)

Figures in parentheses indicate per cent of control

$P < 0.001$  compared to \*control and \*\*PMLE

PMLE, polymorphus light eruption

## Discussion

At present there is considerable interest in free radical-mediated damage in humans following exposure to environmental agents/factors. However, there is no consensus regarding the best quantitative indices of environmental-induced oxidative stress and the effect of antioxidant interventions<sup>25</sup>. A study on the effect of antioxidant therapy (vitamin E) in environmental ultraviolet radiation induced PMLE therefore appeared to be of interest.

High levels of serum MDA in PMLE cases indicate that in this UV induced photodermatitis lipid peroxidation occurs as a result of oxidative stress (OFR generation) like in other such environmental stressors<sup>26</sup>. The extent and severity of the disease was also found to associate with the serum MDA level ( $r=+0.91$ ,  $P<0.001$ ). This was based on comparison between serum MDA level and clinical diagnosis of the patients observed by the dermatologist based on criteria mentioned in the material and methods. This substantiates the hypothesis that enhanced OFR generation may play a role in the pathogenesis of disease. In PMLE cases, incorporation of antioxidant (vitamin E) in treatment was found to be effective. The antioxidant system derangement largely returned to normal (except SOD) following oral vitamin E administration.

The primary defense against oxidative damage of tissues are antioxidant enzymes, *e.g.*: SOD and CAT<sup>25</sup>. SOD plays a role in the destruction of superoxide anion, which is the initial free radical generated among the oxidative radicals to produce hydrogen peroxide. CAT prevents oxidative hazards by catalyzing the formation of water and oxygen from this hydrogen peroxide. Disease may arise from increased exposure to radicals or from impaired efficiency of these protective systems. The results of our study revealed that PMLE, was associated with a decrease in the activity of SOD in erythrocytes with a concomitant increase in CAT activity. Significantly lower activity of SOD in

erythrocytes of PMLE cases may be due to an excessive consumption of SOD as a result of increased free radical production. Interestingly, the effect remains unchanged even after treatment with antioxidant. Although the mechanism is not clear, the finding supports the hypothesis suggesting that the oxidative stress induced by photodermatitis might be contributed by this persisting inhibition of SOD activity. This was also supported by a negative association between SOD activity and MDA level. Further, we found a significant increase in CAT levels in PMLE cases as compared to normal subjects and the extent of elevation of CAT correlate positively with the extent of PMLE ( $r=+0.91$ ,  $P<0.001$ ). This was based on comparison between CAT activity and clinical diagnosis of the patients observed by the dermatologist based on criteria mentioned.

The depletion of blood GSH level in PMLE cases is an index of oxidant burden, because the extent of reduction was negatively related with the blood MDA levels in this study. GSH is the first line of defense against pro-oxidant stress, and this returned to normal after vitamin E administration. It may be inferred that vitamin E helps in sustaining higher levels of GSH. The mechanism by which vitamin E provides an environment for enhanced level of GSH is yet to be ascertained. Moreover, knowing that GR is the enzyme responsible for providing reduced GSH, it is not surprising, to detect an increase in GR activity in PMLE cases as a compensatory mechanism for replenishing the GSH concentration inside the erythrocytes. Further, GPx activity is linked to the GR activity, which supplies reducing equivalents for GPx function. The reduction of GSH in blood in PMLE cases might have resulted from the activity of GPx in reducing lipid hydroperoxides to stable non-radical lipid alcohols, utilizing GSH as the source of reducing equivalents or by the direct utilization of GSH as an antioxidant in terminating free radical reactions initiated by the photodermatitic reactions.

The biological significance of GGT-dependent lipid peroxidation *in vivo* might be multifold. Effects

of photodermatitis on GGT activity are known to be normally balanced by its established role favouring the cellular uptake of precursors of GSH resynthesis. Thus, any increase in GGT activity may influence intracellular synthesis of GSH.

The significant attenuation of lipid peroxidation following vitamin E administration in PMLE cases, confirms that free radical induced alterations can be reversed by antioxidant supplement. We have earlier shown that antioxidants and natural plant products having antioxidant properties can attenuate chemical induced oxidative stress<sup>27,28</sup>.

In conclusion, our results indicated that oxidative stress might be involved in the pathogenesis of photodermatitis in PMLE. Oral vitamin E administration modulated the antioxidant enzymes in a manner that favoured the lowering of lipid peroxidation. Such studies assaying oxidative/antioxidant status during free radical challenge can be used as an index to assess protection against the development of lipid peroxidation in PMLE cases and for assessing success of therapeutic measures. Recently, it has been reported that combining a potent antioxidant with a highly UVA-protective sunscreen is more effective in preventing PMLE, than sunscreen alone and should thus be employed as the prophylaxis of choice for PMLE<sup>29,30</sup>.

The present study also supports incorporating antioxidants in a prophylactic diet of susceptible persons and suggests the use of antioxidant drugs in the treatment protocol of PMLE. Further studies however, are needed to clarify the biochemical and genetic basis of the disease.

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