



## ORIGINAL ARTICLES

### Oxidative stress status in patients with chronic idiopathic urticaria

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#### Abstract

**Aim:** The controversial data related to oxidative stress status in patients with chronic idiopathic urticaria (CIU) have been reported. Therefore, the present study was aimed to contribute to this debate by determining oxidative stress markers along with some trace element levels in patients with CIU.

**Methods:** Twenty-five patients with CIU (10 males, 15 females) and 36 healthy controls were enrolled into the study. Erythrocyte lipid peroxidation status, scavenger enzyme activities and trace element levels were determined.

**Results:** While erythrocyte MDA levels, erythrocyte GSH-Px activities and erythrocyte Zn levels showed no differences between the patient and control groups, a statistically significant decrease and increase were observed in erythrocyte CuZn-SOD activities and Cu levels, respectively, in the CIU patients when compared to those of the controls ( $p < 0.001$  for both of them). **Conclusion:** In conclusion, an oxidative burden which can be relieved by some preserved antioxidant mechanisms seems to be present in patients with CIU even if they are clinically stable and it may probably have a role in the pathogenesis.

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#### Introduction

The role of free oxygen radicals (FOR), occurring in inflammation area, on tissue damage and ethiopathogenesis of various diseases is gradually drawing more attention in medicine. Oxidative stress can be defined as exposure to

increased oxidant or decreased antioxidant capacity.<sup>1</sup> There are some scavenger enzymes such as superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GSH-Px) with antioxidant activity. Some trace elements take part as cofactors in the structure of these enzymes. There are also some non-enzymatic antioxidants like ceruloplasmin and vit-C, vit-E.<sup>2</sup> The most important mechanism in the formation of tissue damage due to free radicals is the peroxidation of lipids.<sup>3</sup> Oxidants launch lipid peroxidation by reacting with multiple unsaturated fatty acids (PUFA). Malondialdehyde

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**Table 1** The values of the parameters studied in the patient and control groups.

	MDA (nmol/ml)	CuZn-SOD (U/gHb)	GSH- Px (U/gHb)	Cu ( $\mu\text{g/ml}$ )	Zn ( $\mu\text{g/ml}$ )
CIU group (n = 25)	37.9 $\pm$ 22.8	258.9 $\pm$ 42.8*	8.00 $\pm$ 1.03	0.82 $\pm$ 0.17*	7.47 $\pm$ 1.62
Control group (n = 36)	39.2 $\pm$ 21.9	404.3 $\pm$ 62.9	8.16 $\pm$ 1.41	0.59 $\pm$ 0.19	7.75 $\pm$ 1.20

\* Statistically significant difference when compared to that of the controls,  $p < 0.001$ .

(MDA), ethene, and pentane are yielded as final products.<sup>4</sup> Therefore, lipid structure of the cell is destroyed, membrane permeability increases, and cellular death occurs.<sup>5</sup>

Inflammatory cells are activated and produce large amounts of FOR in some allergic diseases.<sup>6</sup> It has been suggested that reactive oxygen species (ROS) play an important role in airway inflammation and oxidative stress may also have a role in the pathogenesis of the allergic diseases.<sup>7-11</sup> The conflicting information related to oxidative stress status in patients with chronic idiopathic urticaria (CIU) has been reported.<sup>12-15</sup> The aim of the present study was to search the role of the oxidative stress in the patients with CIU by determining the lipid peroxidation, antioxidant enzyme activities and trace elements in erythrocytes of these patients.

## Materials and methods

Twenty-five patients with CIU, 10 males and 15 females, were enrolled into the study. The study was approved by the local ethics committee of Gulhane School of Medicine. They were symptomatic for more than six weeks, with at least three episodes per week. All well-known causes, such as food and drug allergies, infections, any systemic or neoplastic diseases were excluded. Those with physical and cholinergic urticarias, urticarial vasculitis, and hereditary angio-oedema were not included in the study. Any active treatment was discontinued prior to the study as follows: 30 days for systemic corticosteroids, and 10 days for H<sub>1</sub> and H<sub>2</sub> antihistamines. None of the patients had any previous immunosuppressant therapy history. Patients were not given antihistamines or any drugs which may alter the oxidative marker measurements. The mean age of the patients was 37.36  $\pm$  13.25 years. Patients who applied to Internal Medicine Department outpatient clinic for check-up were included into the study as controls. (n:36, M/F: 19/17). The mean age of the controls was 43.6  $\pm$  16.4 years. Controls had no previous inflammatory disease, nor any drug treatment that could alter the study results.

After an overnight fast, blood samples were drawn from the antecubital vein into the tube containing EDTA to measure the erythrocyte malondialdehyde (MDA) levels, erythrocyte superoxide dismutase (Cu-ZnSOD) activities, erythrocyte glutathione peroxidase (GSH-Px) activities, erythrocyte copper (Cu) and zinc (Zn) levels. Whole blood was separated into plasma and erythrocyte fractions by centrifugation (4000  $\times$  g, 10 minutes) at 4°C. As soon as possible after separation, the erythrocyte fractions were washed three times with saline. Then, erythrocytes were lysed with cold distilled water,<sup>1,4</sup> stored in refrigerator at 4°C for 15 minutes and then their membranes were removed by centrifugating them at 4°C for 30 minutes with 20000  $\times$  g.

Plasma samples and erythrocyte lysate were stored at - 70°C until assay.

Erythrocyte MDA levels along with CuZn-SOD and GSH-Px activities in the erythrocyte lysates were measured by the method of Aydin.<sup>16</sup> MDA levels of erythrocytes were expressed as nmol/ml. CuZn-SOD and GSH-Px activities in erythrocytes lysates were expressed as U/gHb. Copper (Cu) levels and zinc (Zn) levels in the erythrocyte lysates were measured by using flamed atomic absorption spectrophotometry technique. The results were expressed as  $\mu\text{g/ml}$ .

All the data were combined in a common database and statistical evaluation was performed. The difference between the two groups with respect to sex distribution was explored by using Chi-square test. Mann-Whitney U test was used for the comparison of the studied parameters in the control and patient groups. Confidence interval (CI) was taken as 95%,  $p < 0.05$  values were accepted as statistically significant. Results are provided as mean  $\pm$  standard deviation.

## Results

The two groups had no differences from each other with respect to sex distribution ( $\chi^2 = 0.95$ ,  $p = 0.758$ ) and mean age ( $p = 0.130$ ). In terms of the erythrocyte MDA levels; there was no statistically significant difference between the patient and control groups ( $p = 0.319$ ). A statistically significant decrease was observed in erythrocyte CuZn-SOD activities of the patients when compared to that of the controls ( $p < 0.001$ ). However, erythrocyte GSH-Px activities had no difference from those of the controls ( $p = 0.528$ ). Erythrocyte Cu levels were significantly higher in the patients than those of the controls ( $p < 0.001$ ) while erythrocyte Zn levels had no difference ( $p = 0.383$ ) (Table 1).

## Discussion

Skin exposed to different external stimuli chronically may lead to physical urticaria and some of these stimuli produce reactive oxidant radicals.<sup>17</sup> In such circumstances, skin may be exposed to oxidative stress intensively. Some skin irritants are able to generate free radicals and ROS directly through metabolic activation, redox cycling, or other mechanisms and skin application of substances with antioxidant properties prevented skin irritation. It has been suggested that a systemic imbalance exists between peroxidable compounds and the antioxidant system in patients with physical urticaria.<sup>18</sup>

Significant decreases of both CAT and GSH-Px with significant increases in SOD activities in erythrocytes of the patients with physical urticaria were reported previously.<sup>17</sup> In that study,<sup>17</sup> the patients had well-defined physical

urticaria types (solar and cold), in contrast to our patient population with CIU. Additionally, they did not measure erythrocyte MDA levels which would suggest the presence of an oxidative stress injury. In the study of Raho et al.,<sup>12</sup> the activity of SOD and the levels of MDA were observed to be increased in lesional skin samples from CIU patients as compared with skin samples from both non-lesional CIU patients, and healthy controls. However, the authors did not find any difference with respect to the studied parameters between non-lesional CIU patients and control subjects. While their study was conducted in skin samples, our study was performed in erythrocyte lysates. The absence of any difference between their non-lesional CIU patients and healthy controls seems to support the findings from our study including asymptomatic CIU patients without any active skin lesion during the study period.

Superoxide dismutase activity was also reported to be increased in patients with CIU at baseline compared with controls and treatment caused a relevant reduction of SOD activity.<sup>13,19</sup> In these studies, the authors indicated an increase both in SOD activities and ROS levels in plasma and platelets of untreated CIU patients when compared to those of the controls. Since decreased scavenger enzyme activities have been accepted as markers of an oxidative stress<sup>20</sup> decreased ROS levels before treatment would be anticipated in the above-mentioned studies. Furthermore, our study was conducted in erythrocyte lysate from untreated CIU patients, differently from those studies. On the other hand, the antioxidative enzyme activity in plasma (CuZnSOD and MnSOD) and in erythrocytes (CuZnSOD, GSH-Px, and catalase) as well as plasma and erythrocyte level of MDA did not differ significantly between CIU patients and the healthy subjects in the study of Kasperska-Zajac et al.<sup>14</sup> They also stated that there are some differences in the oxidative/antioxidative status assessed in samples of lesional skin, uninvolved skin, and peripheral blood of patients suffering from CIU, as compared with the healthy subjects. In another study of Kasperska-Zajac et al.,<sup>15</sup> activity of CuZnSOD, GSH-Px, and catalase, as well as the MDA level in plasma and erythrocytes did not differ significantly between NSAID-induced patients and the healthy subjects. Although they consider that different samples from the same patients may yield different results about the oxidative/antioxidative status of these patients, they were unable to show any alteration in their study patients with respect to oxidative stress. Our data revealed a statistically significant decrease in CuZnSOD activity in the erythrocytes of the patients with CIU while GSH-Px activity and MDA level in erythrocytes of the same patients were not different from those of the controls (Table 1). Differently from the studies of Kasperska-Zajac et al., our study suggests that there is an oxidative insult which seems to be associated with CIU indicated by decreased SOD activities; but an oxidative stress does not occur due to well-preserved functions of other scavenger enzymes. Nevertheless, no clear-cut conclusions can be obtained from our data about the oxidative stress status of the CIU patients.

In vitro studies have suggested that, similar to iron, Cu acts as a catalyst in the formation of ROS that can lead to oxidative stress and destructive lipid peroxidative damage.<sup>21</sup> Cu-accelerated lipid peroxidation in human

erythrocytes has also been reported.<sup>22</sup> The presence of an oxidative insult in CIU cases is suggested by the data from previous<sup>12-15,17,19</sup> and present studies. Our observation of the statistically significant higher levels of erythrocyte Cu in CIU patients than those of the controls may suggest that Cu may be involved in the oxidative status process of these patients.

In conclusion; it seems there is an oxidative insult in the erythrocytes of the patients with CIU. However, the absence of adequate evidence with respect to an increased lipid peroxidation suggests that exposure to oxidative insult can be overwhelmed by some antioxidative defence mechanisms which remain intact.

## Conflict of interest

The authors have no conflict of interest to declare.

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