Erythrocyte oxidant/antioxidant status in essential hyperhidrosis

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Abstract

Essential Hyperhidrosis is a disorder of excessive, bilateral, and relatively symmetric sweating occurring in the axillae, palms, soles, or craniofacial region without obvious etiology. Nitric oxide may play a physiological part in the production and/or excretion of sweat in skin eccrine glands. Tempol, a SOD mimetic, increases the half-life of NO and results in vasodilatation, hypotension, and reflex activation of sympathetic nervous system. Reactive oxygen species (ROS) may directly activate both central and peripheral sympathetic nervous system activity. We assessed the levels of malondialdehyde (MDA), the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) of red blood cells in patients with essential hyperhidrosis (n = 31) compared to age-and sex-matched healthy controls (n = 28). Erythrocyte activities of SOD and level of MDA were detected significantly higher (p = 0.020, p = 0.004 and respectively) and activities of CAT and GSH-Px were significantly lower (p = 0.0001, p = 0.0001 respectively) in patients than controls. Our results support the hypothesis that oxidative damage resulting from increased ROS production along with insufficient capacity of antioxidant mechanisms may be involved in pathogenesis of EH. (Mol Cell Biochem 290: 131–135, 2006)

Key words: essential hyperhidrosis, catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde

Introduction

Essential hyperhidrosis (EH) is a disorder of excessive, bilateral and relatively symmetric sweating occurring in the axillae, palms, and soles [1]. This condition has a reported prevalence of 2.8% of the population and is associated with significant psychosocial morbidity [2]. The pathophysiology of focal primary hyperhidrosis is poorly understood, but believed to be associated with complex dysfunction of autonomic nervous system, also involving parasympathetic system rather than only a generalized sympathetic over activity [3]. Either thermal stimuli or higher cortical stimuli can activate autonomies that affect sweat of the axillae, face, palms, or soles. The pattern of stimulation can be peculiar to an individual’s inherited dysfunction of the autonomic system. For example, someone with palmar sweating may suffer episodic sweating with either social stress or high ambient temperature. In addition, experts believe that hyperhidrosis is not a neuropsychiatric condition. Recent evidence suggests that hyperhidrosis has a familial component, further suggesting a genetic basis for the condition [1].
The hypothesis that reactive oxygen species (ROS) may play an etiological role in EH remains unknown. In the English literature, there is no study dealing with that subject. ROS including superoxide anion radical (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1O$_2$) and nitric oxide (NO) can cause cellular injury when they are generated excessively or when the enzymatic and non enzymatic antioxidant defense systems are impaired. A number of oxygenated compounds, particularly aldehydes including malondialdehyde (MDA), are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids (PUFAs). Therefore, assessment of thiobarbituric acid reactive substances (TBARS) or 4-hydroxynonenal is probably the most commonly applied method for the measurement of lipid peroxidation [4].

Superoxide dismutase (SOD) is a potent protective enzyme that can selectively scavenge O$_2^-$ by catalyzing its dismutation to H$_2$O$_2$ and molecular oxygen (O$_2$). The other antioxidant enzymes, glutathione peroxidase (GSH-Px) and catalase (CAT), catalyze the conversion of H$_2$O$_2$ to water. Assessment of the activities of these free radical scavenging enzymes in erythrocyte may help to understand the changes in antioxidative status in EH [5].

The hypothesis is that the imbalance between oxidant and antioxidant systems might be involved in the pathophysiology of EH like other skin diseases such as psoriasis, acne, urticaria, contact dermatitis, etc. [6]. Therefore, the aim of the present study was to determine the activities of antioxidant enzymes and the lipid peroxidation end product levels in erythrocyte of EH patients and to compare with healthy controls.

### Materials and methods

This study was conducted by the collaboration of the Department of Dermatology, Medical Faculty of Afyon Kocatepe University and the Department of Medical Biology and Genetics, Medical Faculty of Suleyman Demirel University and approved by the ethical committee of Afyon Kocatepe University Medical School. Also, a complete description of the study was given to each patient and to control subjects and written informed consent was obtained from all subjects.

#### Patients

The patients of the study group were fulfilled the following diagnostic criterias; Focal, visible, excessive sweating of at least 6 months duration without apparent cause with at least two of the following characteristics: Bilateral and relatively symmetric, impairs daily activities, frequency of at least one episode per week, age of onset less than 25 years, positive family history, cessation of focal sweating during sleep [1]. We undertook the history and physical examination together with laboratory tests to exclude other known causes of hyperhidrosis. Thirty-one patients with essential hyperhidrosis were included in this study. Control group is consisted of twenty-eight age- and sex-matched healthy subjects. The patients and control subjects were also matched according to smoking (Table 1).

#### Sample collection and preparation

Fasting blood samples of the study and control subjects (2 ml) were taken from cubital vein into heparinized tubes in the morning, to prepare red blood cell (RBC) sediment. After immediate centrifugation (1000 $\times$ g for 10 min at 4 °C), plasma was removed. After buffy coat was separated carefully, RBC sediment was washed three times with 10-fold isotonic NaCl. At the end of washing, RBC sediment was treated with fourfold ice-cold deionized water to obtain the hemolyzate.

#### Determination of superoxide dismutase activity in erythrocyte

Total (Cu–Zn and Mn) superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to the method of Sun et al. [7]. The principle of the method is based on the inhibition of NBT reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per gram (Ug$^{-1}$) Hb.

#### Determination of catalase activity in erythrocyte

Catalase (CAT, EC 1.11.1.6) activity was determined by the method of Aebi [8]. The principle of the assay is based on

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**Table 1. General characteristics of study groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperhidrosis</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>31</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>23.7 ± 1.6</td>
<td>24.7 ± 1.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>12/19</td>
<td>13/15</td>
<td>N.S.</td>
</tr>
<tr>
<td>Smoking (±)</td>
<td>6/25</td>
<td>2/26</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S.: non significant. Values are given as the means ± SEM.