Evaluation of tissue levels of glutathione S-transferases (GST) isoenzymes in patients with discoid lupus erythematosus

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Abstract

Aim: Cutaneous lupus erythematosus (CLE) has a multifactorial pathogenesis involving genetic and environmental triggers and congenital and acquired immune response. The aim of this study was to investigate the effects of Glutathione S-Transferases (GST) isoenzymes including GSTT1, GSTM1, and GSTP1 in the CLE patients with an etiology of solar radiation exposure.

Material and Methods: Paraffin-embedded skin biopsy sections from the patients were stained by immunohistochemical methods. The results were evaluated under a light microscope by a pathologist. The pattern, localization, and distribution of the immunohistochemical staining were recorded for each patient. Staining of the nucleus or cytoplasm was considered as positive staining. The accuracy of staining was determined based on the intensity and percentage of staining.

Results: No significant difference was found between the patient and control groups regarding staining intensity. In terms of staining percentage, the prevalence of GSTP1-3 genotype was significantly lower in the patient group compared to the control group (25% vs. 63.33%) (p=0.002).

Conclusion: No significant difference was observed in the staining intensity of GSTP1, GSTT1, and GSTM1 between the patient and control groups and the staining percentage in some genotypes was even higher in the control group compared to the patient group.

Keywords: Glutathione S-Transferases; Discoid Lupus; Solar Radiation.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic disease of unknown etiology, characterized by multiple autoantibodies. Cutaneous manifestations of SLE can be seen in 85% of patients during the course of the disease (1). Cutaneous lupus erythematosus (CLE) consists of three variants including subacute, chronic, and other forms. Of these, the chronic form is found in up to 80% of all cases with CLE, with its most common subset being discoid lupus erythematosus (DLE) (2). CLE has a multifactorial pathogenesis, in which genetic and environmental triggers and congenital and acquired immune response play a key role. Although the exact mechanism of CLE remains unclear, ultraviolet (UV) radiation exposure, T-cell dysregulation, B-cell abnormalities, oxidative stress, and dendritic cell activation have been implicated in numerous studies (3-5).

Aerobic organisms are protected from oxygen toxicity by an antioxidant defense system consisting of enzymatic and non-enzymatic components. Oxidative stress is defined as increased production of reactive oxygen species (ROS) and decreased antioxidant defense (6). Glutathione S-transferases (GST) are a diverse family of enzymes that play a key role in the detoxification of ROS products (7). GST are divided into 8 subgroups based on their immunological properties, amino acid sequences, and isoelectric points: alpha, mu, phi, theta, kappa, zeta, sigma, and omega (8). Solar radiation exposure, particularly UVB
radiation exposure, has numerous deleterious effects on the skin. UVB radiation to skin is known to induce ROS, thereby causing reactions such as sunburn, cutaneous photo aging and skin cancer. GST have been shown to provide protection against UV and to prevent membrane lipid peroxidation and DNA damage caused by ROS (9).

In this study, we aimed to investigate the effects of GST isoenzymes including GSTT1, GSTM1, and GSTP1 in the DLE patients with an etiology of solar radiation exposure.

MATERIAL and METHODS

The study was conducted at Yuzuncu Yil University Medical School Dermatology and Pathology departments. The study protocol was performed in accordance with the Declaration of Helsinki revised in 2013 and was approved by the local ethics committee. The retrospective study included 56 patients with a prediagnosis of DLE and 30 age- and gender-matched controls. A 3- or 4-cm skin punch biopsy was performed in each patient and the diagnosis of DLE was confirmed by histopathologic and immunofluorescent examinations. Histopathologic criteria included presence of orthokeratotic hyperkeratosis, follicular plugging, basal cell degeneration, epidermal atrophy, dyskeratosis, thinning of the basal membrane, and perivascular and periadnexal mononuclear cell infiltration. The immunofluorescence criteria included presence of granular deposition of immunoglobulin G and C3 at the dermoepidermal joint. Paraffin-embedded skin biopsy sections from the patients were stained by immunohistochemical methods. The results were evaluated under a light microscope by a pathologist. The pattern, localization, and distribution of the immunohistochemical staining were recorded for each patient. Staining of the nucleus or cytoplasm was considered as positive staining. The accuracy of staining was determined based on the intensity and percentage of staining. The intensity of staining was scored using the following scale: no staining, 0; weak staining, 1+ (less than 10% of the cells); moderate staining, 2+ (10%-70% of the cells); and strong staining, 3+ (>70% of the cells).

Statistical Analysis

Data were analyzed using SPSS for Windows version 17.0 (Chicago: SPSS Inc. IBM Corp.). Descriptive statistics were expressed as frequencies and percentages. Categorical variables were compared using Pearson’s Chi-square test. A p value of <0.05 was considered significant.

RESULTS

No significant difference was found between the patient and control groups regarding staining intensity. In terms of staining percentage, the prevalence of GSTT1-0 genotype was significantly higher in the patient group compared to the control group (94.64% vs. 90%) (p=0.027), whereas the prevalence of GSTP1-3 genotype was significantly lower in the patient group compared to the control group (25% vs. 63.33%) (p=0.002) (Table 1).

Table 1. Staining intensity and percentage in patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1 SI</td>
<td>n: 53</td>
<td>n: 27</td>
<td>0.421</td>
</tr>
<tr>
<td>GSTT1 SP</td>
<td>1: 0</td>
<td>3: 10</td>
<td>0.027</td>
</tr>
<tr>
<td>GSTP1 SP</td>
<td>2: 38</td>
<td>10: 33</td>
<td>0.002</td>
</tr>
<tr>
<td>GSTM1 SI</td>
<td>1: 13</td>
<td>11: 36</td>
<td>0.135</td>
</tr>
<tr>
<td>GSTM1 SP</td>
<td>2: 27</td>
<td>16: 53</td>
<td>0.902</td>
</tr>
</tbody>
</table>

DISCUSSION

The results indicated that no significant difference was observed in the staining intensity of GSTP1, GSTT1, and GSTM1 between the patient and control groups and the staining percentage in some genotypes was even higher in the control group compared to the patient group. The GST isoenzymes have been analyzed in numerous dermatological diseases and the studies have reported different findings. Cho et al., for instance, found an association between atopic dermatitis and GSTM1-0 genotype (10). Tursen et al. reported that the patients with Behçet’s disease showed a higher prevalence of GSTM1-0 genotype (11). Solak et al. revealed that there was no association between GSTM1 and GSTT1-0 genotypes in terms of psoriasis susceptibility (12). Henderson et al. demonstrated that GSTP may lead to alteration in skin cancer susceptibility in mice and may also play a key role in skin cancer susceptibility in humans (13). In our study, we found that the tissue levels of GST did not change in DLE, which is a well-known skin disease. Accordingly, GST does not appear to be an important parameter in DLE patients.

Literature indicates that GST isoenzymes have also been investigated in skin diseases with an etiology of solar radiation exposure. Millard et al. showed that GSTP1 had a protective effect against polymorphic light eruption, also noting that oxidative stress plays an important role in the pathogenesis of polymorphic light eruption (14). Guarneri et al. found that the patients with GSTT1 polymorphism...
showed susceptibility to solar keratosis and concluded that GSTT1 polymorphism can be a significant indicator of susceptibility to skin cancers (15). Aly et al. showed that GSTM1/GSTT1 polymorphisms may induce susceptibility to generalized vitiligo, which elucidates the relations between vitiligo, oxidative stress, and genetics (16). Unlike previous studies, the present study found that the tissue levels of GST isoenzymes did not change between the DLE patients and the control subjects. This finding suggests that there is no association between solar radiation exposure and GST isoenzymes. Literature reviews indicate that there are a limited number of studies reporting on GST isoenzymes in SLE. Kang et al. evaluated SLE in a Korean population and reported that the GST isoenzymes provided protection against the development of SLE by inactivating ROS that result in damage to some tissues and DNA. The authors also noted that the patients with GSTM1-0 genotype had a lower frequency of hematological diseases (17). Audemard-Verger et al. evaluated patients undergoing cyclophosphamide treatment due to lupus nephritis and found that the GST polymorphisms were associated with the side effects of cyclophosphamide and the preservation of renal functions. The authors also showed that GST1 induces apoptosis in glomerular cells and leads to accumulation of ROS, thereby leading to decreased detoxification in the kidneys (18). In our study, we evaluated GST isoenzymes in DLE, which is a form of SLE characterized by skin involvement. To our knowledge, this is the first study of its kind in the literature. Moreover, unlike previous studies, the present study found that there was no significant difference in GST isoenzymes between the patient and control groups, which implicates that DLE can also be seen in patients diagnosed with diseases other than SLE.

The study was limited in several ways. First, it had a retrospective design. Secondly, it had a relatively small patient population. Finally, the study did not evaluate all of the GST isoenzymes.

**CONCLUSION**

In conclusion, the tissue levels of GSTT1, GSTM1, GSTP1 did not change in patients with DLE. Further multi-center studies are needed to substantiate our findings.

**Competing interests:** The authors declare that they have no competing interest.

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**Ethical approval:** This work has been approved by the Institutional Review Board

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