Original article

CED
Clinical and Experimental Dermatology

Association of oxidative stress and dynamic thiol-disulphide homeostasis with atopic dermatitis severity and chronicity in children: a prospective study

P. Uysal,[1] S. Avcil,[2] S. Neşelioğlu,[3] C. Biçer[3] and F. Çatal[4]

Departments of [1]Pediatric Allergy and Immunology and [2]Child and Adolescent Psychiatry, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey; [3]Department of Clinical Biochemistry, Faculty of Medicine, Yildirim Beyazid University, Ankara, Turkey; and [4]Department of Pediatric Allergy and Immunology, Faculty of Medicine, Inonu University, Malatya, Turkey

doi:10.1111/ced.13219

Summary

Background. Oxidative stress (OS) has an important effect on the pathogenesis of atopic dermatitis (AD). Thiols are antioxidants that regulate intracellular redox metabolism and protect keratinocytes against OS damage in the stratum corneum.

Aim. To investigate dynamic thiol-disulphide homeostasis (dTDH) as a novel OS parameter in children with AD, and its relationship with disease severity and chronicity.

Methods. Severity of AD was determined by using the instruments SCORing Atopic Dermatitis (SCORAD) and Eczema Area And Severity Index (EASI) upon enrolment in the study ($SCORAD_1$ and $EASI_1$) and after 1 year ($SCORAD_2$ and $EASI_2$). Native thiol, total thiol and disulphide levels were measured as novel OS parameters, and the ratios of disulphide/native thiol, disulphide/total thiol and native/total thiol were calculated as dTDH.

Results. In the AD group, the serum disulphide level and the ratios of disulphide/native thiol and disulphide/total thiol were significantly lower than in healthy controls ($P = 0.01$, $P < 0.01$ and $P < 0.01$, respectively). There was no significant association between OS parameters and disease severity ($P > 0.05$). $SCORAD_2$ and $EASI_2$ were positively correlated with disulphide/native thiol ratio ($r = 0.29$, $P < 0.03$ and $r = 0.35$, $P < 0.01$, respectively), whereas they were negatively correlated with the native/total thiol ratio ($r = -0.30$, $P = 0.02$ for both).

Conclusions. Both OS and impaired dTDH were found to be related to childhood AD. None of the OS parameters was associated with AD severity. dTDH is a possible diagnostic tool to predict AD chronicity.

Introduction

Atopic dermatitis is a chronic, pruritic and relapsing inflammatory skin disease.[1] Although the pathophysiological mechanisms of atopic dermatitis (AD) are complex and have not been entirely defined, many observations have shown the profound influence of oxidative stress (OS) in the pathogenesis of this disease.[2]

OS is the result of reactive oxygen species (ROS) overproduction or of inadequate antioxidant defence mechanisms, leading to disruption of the oxidant–antioxidant balance towards the oxidants.[3] OS is one of the most important factors in AD pathophysiology. OS can damage keratinocyte DNA through lipid oxidation, as well as disrupt barrier function, enhance proinflammatory cytokine production and activate naive T cells and cellular dermal infiltration, and consequently aggravate AD lesions.[4,5]

Correspondence: Dr Pinar Uysal, MD, Department of Pediatric Allergy and Immunology, Faculty of Medicine, Adnan Menderes University, Aydin, 09200, Turkey
E-mail: druysal.pinar@gmail.com

Conflict of interest: the authors declare that they have no conflicts of interest.

Accepted for publication 23 August 2016

Thiols are powerful antioxidants that scavenge ROS, regulate intracellular redox metabolism and protect keratinocytes against the consequences of oxidative modification in the stratum corneum.[6] In skin, mature dendritic cells (DCs) are the major source of thiols, which are essential for proliferation of naive T cells. In allergic skin diseases, thiol-containing molecules have been found to be over-expressed in the epidermis at positive allergen patch test sites.[7] Thiols have also been demonstrated to cause downregulation of Th2 polarization in a dose-dependent manner.[8]

Total thiols are composed of both intracellular and extracellular thiols, and these consist of both reduced and nonreduced thiols; native thiol refers only to nonreduced thiol.[9] In the OS state, the plasma thiol levels tend to reduce because thiol is oxidised to form disulphides. The ratio between the thiol and disulphide parameters indicates the balance of the oxidant–antioxidant status via a continuous reciprocal reaction, executing dynamic thiol/disulphide homeostasis (dTDH),[10] and dTDH plays a particular role in antioxidant protection, signal transduction and enzymatic activity regulation.[11,12] However, to date, no research has been reported in the literature investigating thiol homeostasis in AD. Thus, we aimed to investigate the relationships between dTDH and AD, AD severity and AD chronicity.

Methods

The study protocol (number 2015/588) was approved by the local ethics committee of our institution. Written informed consent was obtained from the caregivers of the participants.

Participants

In total, 60 children aged 12–36 months with recently diagnosed AD with active lesions and 60 sex- and age-matched healthy controls (HCs) were enrolled at the outpatient clinic of the Paediatric Allergy Department at a tertiary referral hospital in the Aegean region of Turkey.

The clinical diagnosis of AD was based on diagnostic criteria published by Williams *et al.*[13] Data on patient demographics were recorded, and blood samples were obtained at the first appointment. Severity of AD was assessed by using two scoring indexes, the SCORing Atopic Dermatitis (SCORAD) scale and the Eczema Area And Severity Index (EASI),[14] which upon enrolment were defined as $SCORAD_1$ and $EASI_1$ for the beginning of the study, and as $SCORAD_2$ and $EASI_2$ for the end of the study.

The following exclusion criteria were used: presence of eczema that did not fulfil the diagnostic criteria[13] for AD; coexistence of severe chronic disease (e.g. liver or kidney diseases, malignancy, diabetes, primary immune deficiency); use of systemic or topical therapies (such as vitamins or anti-inflammatory drugs); and consumption of high-antioxidant diets (which might interfere with the OS) during the 2 weeks prior to study onset.

Measurements

Native thiol, total thiol and disulphide were measured as novel OS parameters, and the ratios of disulphide/native thiol, disulphide/total thiol and native/total thiol were calculated as dTDH. To avoid extraneous variables that might interfere with the study results, all of the participants were advised not to perform heavy exercise prior to blood sampling. Dynamic disulphide bonds (–S–S–) in the serum sample were reduced to native thiol groups (–SH) by $NaBH_4$. The total thiol content was measured using a modification of Ellman reagent. Native thiol content was subtracted from the total thiol content, and half of the obtained difference gave the disulphide bond amount. Parameters were measured using a spectrophotometer (UV-1800; Shimadzu Corp., Kyota, Japan) and an automated analyser (cobas c 501; Roche Diagnostics, Indianapolis, IN, USA), as described in the study conducted by Erel *et al.*[12]

Statistical analysis

The data were analysed using IBM SPSS for Windows; (v18.0; IBM, Armonk, NY, USA). The χ^2 test was used to evaluate qualitative data, while Student *t*-test, ANOVA and Mann-Whitney *U*-test were used to assess quantitative data. Pearson and Spearman rank correlation coefficients were used for correlation analysis. $P < 0.05$ was considered statistically significant. A receiver operating characteristic (ROC) curve was drawn to find the cutoff point for parameters of dTDH in order to evaluate the prediction of AD chronicity.

Results

Demographics

Age and sex were not significantly different between the AD and HC groups ($P > 0.05$) (Table 1). Median

AD duration was 12 months [interquartile range (IQR) 6–23.25]. In children with AD, mean $SCORAD_1$ as 48.57 ± 18.74 (range 13.2–90) and mean $EASI_1$ was 14.6 ± 14.4 (range 0.4–55.7) while median $SCORAD_2$ was 12.45 (IQR: 0–22.87) and median $EASI_2$ was 0.40 (IQR: 0–4.80).

Thiol/disulphide levels

The AD group's serum disulphide level and the ratios of disulphide/native thiol and disulphide/total thiol were found to be significantly lower in patients with AD than in HCs ($P = 0.01$, $P < 0.01$ and $P < 0.01$, respectively) (Table 1).

Association between thiol/disulphide levels and atopic dermatitis

There was no significant difference between mild, moderate and severe AD in terms of native thiol, total thiol or disulphide levels, or in terms of disulphide/native thiol, disulphide/total thiol or native/total thiol ratios (Table 2).

There was a positive correlation between the disulphide/native thiol ratio and $SCORAD_2$ ($r = 0.29$, $P = 0.02$) (Fig. 1a) and EASI2 ($r = 0.35$, $P < 0.01$) (Fig. 1a). However, there was a negative correlation between the native/total thiol ratio and $SCORAD_2$ ($r = -0.30$, $P = 0.02$) (Fig. 1a) and $EASI_2$ ($r = -0.30$, $P = 0.02$) (Fig. 1b).

Table 1 Comparison of demographics and plasma oxidative stress parameters between children with atopic dermatitis and HCs.

	Children with AD ($n = 60$)	HCs ($n = 60$)	*P*
Age, months, median (IQR)	18 (15.5–28)	20.5 (16–29.5)	0.99
Sex, *n* (%)			
Male	40 (66.7%)	39 (65%)	0.99
Female	20 (33.3%)	21 (35%)	
Familial history of allergic diseases* *n* (%)			
Parental	26 (43.3%)	15 (25%)	0.03
Maternal	9 (15%)	6 (10%)	0.41
Paternal	15 (25%)	7 (21.7%)	0.06
Disulphide and thiol			
Native thiol, ng/mL	423.27 ± 66.61	408.84 ± 55.40	0.20
Total thiol, ng/mL	466.80 ± 70.24	455.85 ± 57.44	0.35
Disulphide, ng/mL	2183.0 ± 493.98	2426.10 ± 546.17	0.01
Disulphide/native thiol ratio	5.26 ± 1.32	5.98 ± 1.39	< 0.01
Disulphide/total thiol ratio	4.73 ± 1.06	5.32 ± 1.09	< 0.01
Native thiol/total thiol ratio	0.90 ± 0.05	0.89 ± 0.03	0.22

HCs, healthy controls; IQR, interquartile range. Values are mean ± SD unless otherwise stated. *Allergic rhinitis, asthma or atopic dermatitis. Differences between patients and HCs were tested by χ^2 test, Mann–Whitney *U*-test or Student *t*-test, as appropriate.

Table 2 Comparison of plasma oxidative stress parameters and disease scoring indexes between children with mild, moderate or severe atopic dermatitis.

	Mild AD ($n = 6$)	Moderate AD ($n = 28$)	Severe AD ($n = 26$)	*P*
Disulphide and thiol				
Native thiol, ng/mL	467.53 ± 45.06	412.46 ± 49.72	424.69 ± 82.42	0.06
Total thiol, ng/mL	512.78 ± 51.08	454.56 ± 55.83	469.38 ± 84.11	0.08
Disulphide, ng/mL	2262.50 ± 542.71	2230.08 ± 442.15	2113.96 ± 544.87	0.66
Disulphide/native thiol ratio	4.83 ± 1.00	5.48 ± 1.22	5.12 ± 1.48	0.29
Disulphide/total thiol ratio	4.39 ± 0.82	4.96 ± 1.03	4.57 ± 1.13	0.27
Native thiol/total thiol ratio	0.91 ± 0.01	0.91 ± 0.06	0.90 ± 0.03	0.54
Scoring instruments				
$SCORAD_1$	20.91 ± 3.93	38.0 ± 6.87	66.23 ± 11.89	< 0.001
$EASI_1$	1.08 ± 0.58	6.57 ± 6.17	26.36 ± 13.95	< 0.001
$SCORAD_2$, median (IQR)	5.95 (0–14.57)	0 (0–19.65)	18.25 (0–30.4)	< 0.05
$EASI_2$, median (IQR)	0.1 (0–0.4)	0 (0–2.4)	3.9 (0–12)	0.02

IQR, interquartile range. Values are mean ± SD unless otherwise stated. Differences between patient groups were tested by one-way ANOVA, Tukey and Kruskal–Wallis tests.

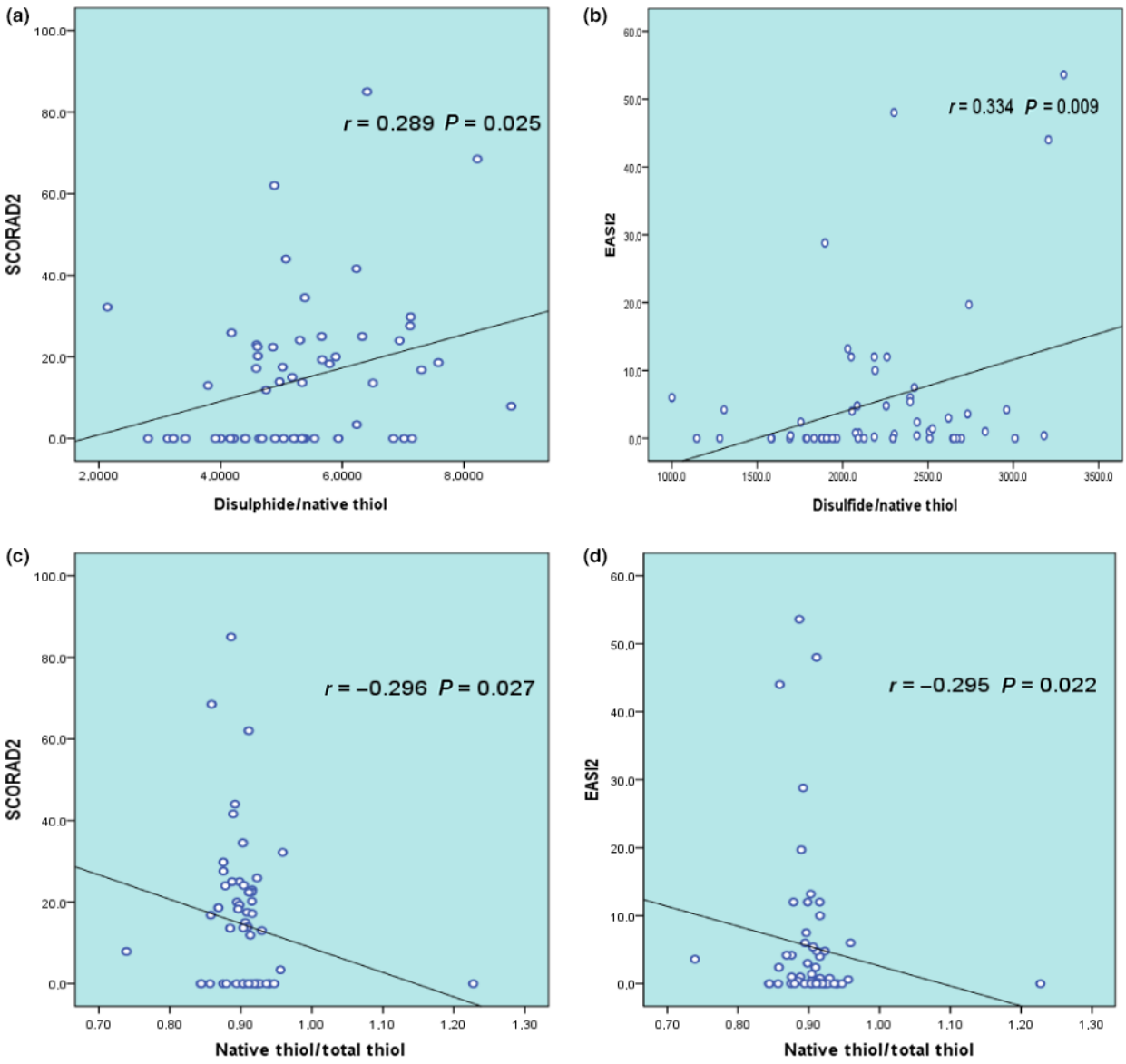


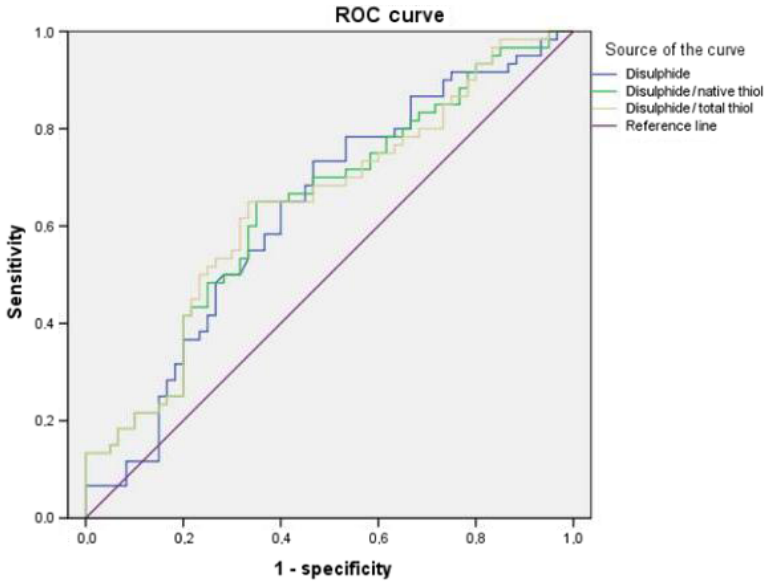
Figure 1 (a,b) Correlation between (a) SCORing Atopic Dermatitis (SCORAD) and Eczema Area And Severity Index (EASI) after 1 year ($SCORAD_2$ and $EASI_2$) and disulphide/native thiol ratio and (c,d) between $SCORAD_2$ and $EASI_2$ and native thio/total thiol ratio.

In the ROC curve, disulphide and disulphide/native thiol and disulphide/total thiol ratios predicted AD chronicity (AUC = 0.63, $P = 0.01$; AUC = 0.64, $P < 0.01$; AUC = 0.64 and $P < 0.01$, respectively) (Fig. 2c,d). The cut-off point was ≥ 1976 ng/mL for disulphide, with a sensitivity of 68.3% and a specificity of 94%; ≥ 5.34 for the disulphide/native thiol ratio, with a sensitivity of 75.6% and a specificity of 97%; and ≥ 4.35 for the disulphide/total thiol ratio, with a sensitivity of 67.1% and a specificity of 92% for predicting AD chronicity after 1 year.

Discussion

The main finding of this study was the presence of decreased levels of serum disulphide and decreased ratios of disulphide/native thiol and disulphide/total thiol in children with AD versus HCs. The OS parameters were not related to disease severity, but dTDH was found to be a good parameter for predicting AD chronicity.

In recent years, a few studies have investigated the possible role of OS in the aetiology of AD. More



Test variable	Area	*P* value	95% confidence interval	
			Lower bound	Upper bound
Disulphide	0.631	0.014	0.530	0.731
Disulphide/ native thiol	0.643	0.007	0.544	0.741
Disulphide/ total thiol	0.644	0.006	0.546	0.743

Figure 2 Receiver operating characteristic (ROC) curve for disulphide/native thiol ratio predicted atopic dermatitis chronicity.

recently, Amin *et al.* and Sivaranjani *et al.* found higher malondialdehyde (MDA) levels but lower levels of antioxidants in patients with AD versus HCs in case–control studies.[15,16] Chung *et al.* found higher serum MDA and lipid peroxidation product levels in preschool children with AD compared with a control group.[17] Omata *et al.* obtained results compatible with ours; they found lower NO synthesis in children with chronic AD.[18] Subsequently, the same researchers reported altered antioxidant defence and increased OS in children with acute exacerbation of AD. In the same study, the authors reported that endogenous NO levels (an OS marker) in children with an acute exacerbation of AD were similar to those of HCs, and that their results were unexpected given the immunopathology. This led to the hypothesis of an impaired NO pathway in AD.[19] In the current study, we found impaired dTDH in children with AD compared with HCs. Taken together, the results suggest that OS or impaired dTDH may be the result of interrupted or blocked antioxidant production or of high consumption of thiols in the OS state in AD pathogenesis. Additionally, proportional reduction in disulphide to thiol levels might be a rebound phenomenon to maintain protective effects by facilitating the neutralization state of ROS.

To our knowledge, our study is the first to investigate the association between thiol homeostasis and the severity of AD. Although we could not demonstrate any association between dTDH and disease severity, both native and total thiol levels were higher in mild AD than in moderate or severe AD. Moreover, the disulphide/native thiol ratio was positively correlated and the native/total thiol ratio was negatively correlated with higher scoring indexes evaluated after 1 year. The results of other studies support our results, with data showing the impact of OS parameters (total antioxidant status, total oxidative status and OS index) on the severity of T cell-mediated skin diseases.[20–22] In addition to regulating antioxidant function, thiols downregulate T helper (Th)2 polarization and Th2-mediated cytokine production in a dose-dependent manner.[8] Thiols enhance T-cell proliferation and downregulation of Th2-mediated cytokines, including interleukin (IL)-4, IL-5 and interferon-γ in human T cells, and decrease production of IgE and IgG4 by B cells.[23] Moreover, thiol depletion in antigen-presenting cells inhibits Th1-mediated cytokines and favours Th2-mediated

responses.[24] We hypothesize that thiols might be either consumed or oxidized to their derivatives to remove excess ROS, and the consequent reduction in thiol levels may cause Th2-skewed inflammatory reactions, which subsequently lead to more severe allergic inflammation.

It is still unknown how Th2-type inflammation and activation of keratinocytes interacts with and leads to chronicity of AD inflammation. Chronic skin inflammation is associated with overproduction of ROS and high levels of products of lipid peroxidation. OS can in turn influence immune cell activation, differentiation and survival; it also affects the propagation of the inflammatory response and the development of chronicity.[25] There are no studies in the literature evaluating the role of thiol homeostasis in the prediction of AD prognosis. We propose that OS may be an intrinsic mediator of amplification and chronicity in allergic skin inflammation, and dTDH seems be a key diagnostic parameter for predicting the chronicity of childhood AD.

Strengths and limitations

The strengths of the present study were the evaluation of the children by the same paediatric allergist and the use of two different scoring measures to evaluate AD severity, which decreased the subjective evaluation approach to disease severity and increased the reliability of the study results. With the aid of a novel, fully automated assay invented by Erel *et al.*, the dTDH was determined by a more reproducible, practical and inexpensive method than the generally accepted but time-consuming, costly and complicated conventional assays.[12] Measurement of dTDH (as opposed to measurement of isolated OS parameters and total oxidant or antioxidant status) allows more in-depth study of the dynamic OS mechanisms in childhood AD.

This study also had some limitations. The potential associations between dTDH and skin infections and/or colonization, filaggrin (FLG) mutation and treatment responses could not be evaluated. Because dTDH was evaluated by serum sampling, our results might be affected, with the possibility that inflammatory cells home into chronically inflamed skin.

Conclusion

We have shown that impaired dTDH is related to childhood AD, and that disulphide, disulphide/native thiol ratio and disulphide/total thiol ratio have predictive value as diagnostic tools for evaluating the chronicity of childhood AD. Because it could be impossible to explain all of the OS mechanisms solely via dTDH, this study provides only a new look inside the role of dynamic oxidant–antioxidant homeostasis in AD pathogenesis. Additional *in vivo* and *in vitro* studies are required to confirm the preliminary study results and to better understand the role of dTDH in the pathogenesis of AD. After an understanding of the exact OS mechanisms in AD has been achieved, development of novel pharmaceutical antioxidant agents may add valuable contributions to the standard treatments currently being used, including moisturizing lotions and creams, topical corticosteroids and calcineurin inhibitors. Antioxidant supplementation may help to prevent AD or aid to mitigate disease progression in the future.

Acknowledgements

We thank M. Alişik (Department of Clinical Biochemistry, Faculty of Medicine, Yildirim Beyazid University) for his contribution to the sample analysis. Special thanks to Ö. Erel for his contribution of financial support to the study via the Department of Clinical Biochemistry (Faculty of Medicine, Yildirim Beyazid University).

What is already known about this topic?

- OS has important effects on the pathogenesis of AD.
- Thiols are antioxidants that regulate intracellular redox metabolism in the stratum corneum.

What does this study add?

- With the aid of a novel, fully automated assay, dTDH level was determined to understand the dynamic OS mechanisms in AD.
- OS and impaired dTDH were found to be related to childhood AD.
- dTDH could be a possible diagnostic tool to predict AD chronicity.

References

1 Boguniewicz MLD. Atopic dermatitis. In: *Middleton's Allergy Principles and Practice*, 7th edn (Adkinson NK, Bochner BS, Burks AW *et al.*, eds). Philadelphia: Elsevier Inc., 2014: 540–65.

2 Ji H, Li XK. Oxidative stress in atopic dermatitis. *Oxid Med Cell Longev* 2016; **2016**: 1–8.
3 Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; **35**: 1147–50.
4 Wright RJ, Cohen RT, Cohen S. The impact of stress on the development and expression of atopy. *Current Opin Allergy Clin Immunol* 2005; **5**: 23–9.
5 Pastore S, Korkina L. Redox imbalance in T cell-mediated skin diseases. *Mediators Inflamm* 2010; **2010**: 1–9.
6 Slominski AT, Kleszczyński K, Semak I *et al.* Local melatoninergic system as a protector of skin integrity. *Int J Mol Sci* 2014; **15**: 17705–32.
7 Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new. *J Eur Acad Dermatol Venereol* 2003; **17**: 663–9.
8 Hasan AA, Ghaemmaghami AM, Fairclough L *et al.* Allergen-driven suppression of thiol production by human dendritic cells and the effect of thiols on T cell function. *Immunobiology* 2009; **214**: 2–16.
9 Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med* 2013; **65**: 244–53.
10 Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med* 2009; **47**: 1329–38.
11 Groitl B, Jakob U. Thiol-based redox switches. *Biochim Biophys Acta Proteins Proteomics* 2014; **1844**: 1335–43.
12 Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014; **47**: 326–32.
13 Williams HC, Burney PG, Strachan D, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. II. Observer variation of clinical diagnosis and signs of atopic dermatitis. *Br J Dermatol* 1994; **131**: 397–405.
14 Kunz B, Oranje AP, Labrèze L *et al.* Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997; **195**: 10–19.
15 Amin MN, Liza KF, Sarwar MS *et al.* Effect of lipid peroxidation, antioxidants, macro minerals and trace elements on eczema. *Arch Dermatol Res* 2015; **307**: 617–23.
16 Sivaranjani N. Role of reactive oxygen species and antioxidants in atopic dermatitis. *J Clin Diagnostic Res* 2013; **7**: 2683–5.
17 Chung J, Oh SY, Shin YK. Association of glutathione-S-transferase polymorphisms with atopic dermatitis risk in preschool age children. *Clin Chem Lab Med* 2009; **47**: 1475–81.
18 Omata N, Tsukahara H, Ito S *et al.* Increased oxidative stress in childhood atopic dermatitis. *Life Sci* 2001; **69**: 223–8.
19 Tsukahara H, Shibata R, Ohshima Y *et al.* Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. *Life Sci* 2003; **72**: 2509–16.
20 Bakry OA, Elshazly RMA, Shoeib MAM, Gooda A. Oxidative stress in alopecia areata: a case-control study. *Am J Clin Dermatol* 2014; **15**: 57–64.
21 Kaur S, Zilmer K, Leping V, Zilmer M. Allergic contact dermatitis is associated with significant oxidative stress. *Dermatol Res Pract* 2014; **2014**: 1–7.
22 Emre S, Metin A, Demirseren DD *et al.* The association of oxidative stress and disease activity in seborrheic dermatitis. *Arch Dermatol Res* 2012; **304**: 683–7.
23 Jeannin P, Delneste Y, Lecoanet-Henchoz S *et al.* Thiols decrease interleukin (IL) 4 production and IL-4 induced immunoglobulin synthesis. *J Exp Med* 1995; **182**: 1785–92.
24 Peterson J, Herzenberg L, Vasquez A, Waltenbaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci USA* 1998; **95**: 3071–6.
25 Sorokin L. The impact of the extracellular matrix on inflammation. *Nat Rev Immunol* 2010; **210**: 712–23.

