A Predictor of Oxidative Stress in the Children with Measles: Thiol–Disulfide Homeostasis

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What is already known on this topic?
- Oxidative stress can be defined as the total burden resulting from many reactions at a molecular level, which is harmful to the body.
- Oxidative stress index is an indicator of the degree of oxidative stress.
- The oxidative stress index is the percentage of the total oxidant status level/total antioxidant status level ratio.

What this study adds on this topic?
- Thiol–disulfide balance is associated with oxidative stress in various diseases.
- Measles is a highly contagious disease that can cause fatal complications in children. Therefore, measuring oxidative stress is important in measles.
- The results of this study demonstrate that increased oxidative stress in measles can be detected using the thiol–disulfide balance.

ABSTRACT

Objective: Measles is an infectious disease, in which oxidative stress increases. Thiols are an antioxidant substance which play a critical role in programmed cell death, detoxification, and regulation of cellular enzymatic activity, and the thiol–disulfide balance is associated with some diseases. The purpose of this study was to evaluate the thiol–disulfide balance in children with measles.

Materials and Methods: This descriptive study included case and control groups. The plasma total oxidant status level was measured using the Erel method, and the groups were compared. Before the study, informed consent was obtained from patients and Ethics Committee approval was provided (No:17/Session:05, Date: May 2019). The Pearson’s and Fisher’s chi-square tests were applied in the comparisons of categorical data, and independent t-test/Mann–Whitney U tests were used to compare the patient and the control groups.

Results: There were no significant differences between the patient-control groups in terms of age and gender (P > .05). The total antioxidant status value was significantly lower, and the total oxidant status and oxidative stress index values were significantly higher (P < .05) in the patient group compared to the control group. Native thiol, total thiol, and native thiol/total thiol percentage values were significantly lower, and the disulfide, disulfide/native thiol, and disulfide/total thiol percentage values were significantly higher (P < .05) in the patients compared to the controls.

Conclusions: The detection of oxidative stress in patients with measles is important, and these results show the possibility of using the thiol/disulfide homeostasis and oxidative stress index values as biomarkers of oxidative stress in patients with measles.

Keywords: Child, measles, oxidative stress, thiol–disulfide

INTRODUCTION

Oxidative stress occurs in response to oxidative damage caused by the imbalance between free radicals, other oxidants, and the antioxidant defense system. In healthy individuals, free oxygen radicals are in balance with the antioxidant system.1 Reactive oxygen species (ROS) make up the majority of active oxides and can affect almost all cell components.1 In contrast, antioxidants are protective mechanisms that limit or partially repair oxidative damage caused by free oxygen radicals in an organism.2 During oxidative stress, the antioxidant defense may become weakened and/or free oxygen radical production may increase.3,4

Total oxidant status (TOS) is used to measure the body’s overall oxidation, while total antioxidant status (TAS) is used to measure the body’s overall antioxidant status. The oxidative stress index (OSI), which is the ratio of TOS to TAS, can be a more precise index of oxidative
stress. In summary, TOS, TAS, and OSI are oxidative stress parameters used to evaluate the oxidative stress status.1

Some substances in the human body act as antioxidants. The thiol group, also known as mercaptan, is one of the antioxidant groups. Thiols are organic compounds composed of a sulfur atom attached to a carbon atom and a hydrogen atom containing a sulfhydryl group (–SH).5-7 Thiols contribute to a large proportion of the total antioxidants in the human body by acting as fast electron acceptors, and they play an important role in defense against ROS.6-10 They also have a critical role in programmed cell death, detoxification, antioxidant protection, and the regulation of cellular enzymatic activity.6,11

Measuring the thiols in serum is an indirect indicator of antioxidant defense.5,12 Plasma total thiol, natural thiol, and disulfide levels are increasingly being used in routine clinical diagnosis and in the monitoring of various human diseases and metabolic disorders.10 For example, an impaired thiol–disulfide balance has been associated with various diseases, such as thalassemia, hepatitis–B, diabetes mellitus, and familial Mediterranean fever.13-16

Measles is an infectious disease that is generally observed in childhood. Some fatal complications, such as subacute sclerosing panencephalitis (SSPE), can develop in infected children younger than 2 years.17 Oxidative stress has been shown to increase in children with measles with a breakdown of the oxidant/antioxidant balance system.18,19 In literature, oxidative injury has been reported in patients with SSPE,20,21 but there has been no explanatory study about the thiol–disulfide balance in measles. Therefore, the aim of this study was to determine the thiol–disulfide balance in children with measles, to evaluate how TAS, TOS, and OSI change in measles, and thereby contribute to the diagnosis and treatment methods that can be developed through the antioxidant pathway.

MATERIALS AND METHODS

Design and Study Population
This retrospective descriptive study was conducted in the pediatric department of a state university in Turkey. The case group included 43 children with measles and a control group was comprised of fully healthy children, aged 0-10 years, who presented at the pediatrics clinic for a check-up, and had no chronic illness or infections.

Inclusion Criteria
Children aged 0-10 years, with no chronic disease, who had clinical findings of measles (e.g., fever >39.5°C, cough, conjunctivitis, Koplik’s spots, and diffuse maculopapular rash) and IgM positivity (>1.1 RU/mL) detected by ELISA (using the EUROIMMUN microplate ELISA method with a Pfizer brand kit), were accepted as the measles positive participants.

Exclusion Criteria
Children older than 10 years, with a chronic disease, and/or with negative IgM results were excluded from the study.

Data Collection
Blood samples of the patients and the control group subjects were centrifuged at 3000 rpm for 5 minutes. The obtained serum samples were stored at −80°C until analysis.

Measurement of Parameters
Thiol/disulfide homeostasis tests were applied using the spectrophotometric method described by Erel and Neselioglu.12 The disulfide bonds were first reduced to form free functional thiol groups containing sodium borohydride. To protect 5,5’-dithiobis-(2-nitrobenzoic) acid (DTNB), unused reducing sodium borohydride was consumed with formaldehyde and removed. All thiol groups reduced through the reaction with DTNB (including disulfide, native thiol, and total thiol groups) were determined. Finally, disulfide amounts were calculated as disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol percentages.

Plasma TOS levels were measured using the Erel12 TOS method in which oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to a ferric ion. The oxidation reaction was enhanced by glycerol molecules, present in abundance in the reaction medium. The ferric ion formed a colored complex with xylene in an acidic medium. The color intensity, which can be measured spectrophotometrically, was related to the total amount of oxidant molecules in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in micromoles per liter of hydrogen peroxide equivalent (μmol H₂O₂ eq/L).

The plasma total TAS levels were measured using the Erel method.23 The fundamental principle of the assay is to incubate 2,2’-casino-di-3-ethylbenzthiazoline sulfonate (ABTS) with H₂O₂ to produce the ABTS+ radical cation, which has a relatively stable blue-green color and is measured at 600 nm. Antioxidants in the added serum caused bleaching of this color to a degree proportional to their concentrations. The TAS value was expressed as μmol Trolox eq/L.

The ratio of the TOS to TAS was accepted as the OSI in this study. To perform this calculation, the obtained TAS unit was converted to μmol/L and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H₂O₂ eq/L)/TAS (μmol Trolox eq/L).24

Statistical Analysis
Data obtained in the study were analyzed statistically using the Statistical Package for Social Sciences, version 20.0 software (SPSS Inc.; Chicago, IL, USA). The conformity of variables to normal distribution was evaluated using the Kolmogorov–Smirnov
and TOS and OSI were significantly higher \(P < .001\). Native thiol \(P = .005\), total thiol \(P = .046\), and native thiol/total thiol percentage \(P < .001\) were significantly lower in the patient group compared to the control group. Disulfide \(P = .03\), disulfide/native thiol percentage \(P < .001\), and disulfide/total thiol percentage \(P < .001\) were determined to be significantly higher in the patient group than in the control group (Table 2). No significant difference was found in oxidative status between hospitalized and non-hospitalized patients \(P < .05\). The mean ± SD values for the independent samples t-test and median values (with minimum and maximum values) for the Mann–Whitney U test are shown in Table 2.

**DISCUSSION**

Endogenous oxidation reactions, which take place in the intra-leukocyte microbial killing mechanism in the host defense system, are actively used by the immune system in infectious diseases. Measles is an infectious disease that can be caused by oxidative stress on the immune system and may have serious complications with ROS emerging as a result of various oxidative reactions. Reactive oxygen species can damage normal tissues if not controlled by the antioxidant defense system. Previous studies have shown that treatments especially which increase the antioxidant potential also greatly improve the clinical picture of infectious diseases.\(^{25,26}\) Therefore, those results also suggest that antioxidant agents may be important in controlling active measles disease.

Thiols are physiological free radical cleaners that destroy ROS with enzymatic or non-enzymatic mechanisms. They regulate intracellular redox metabolism and protect cells against the results of oxidative changes.\(^{27}\) Dynamic thiol/disulfide homeostasis has an antioxidant impact, which contributes to the regulation of detoxification, signal transduction, apoptosis, enzymatic activity, transcription factors, and the cellular signaling mechanism.\(^{11,28,29}\) Therefore, thiols are the antioxidants that are consumed first during oxidative stress, and as such, the determination of the plasma thiol levels provides important

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**Table 1. Patient Group Clinical Finding Parameters**

| Clinical Finding Parameters | Number of Patients (n = 43) | % Value |
|----------------------------|-----------------------------|---------|
| Fever                      | 41                          | 95.3    |
| Cough                      | 42                          | 97.7    |
| Nasal flow                 | 35                          | 81.4    |
| Conjunctivitis             | 26                          | 60.5    |
| Otitis media               | 2                           | 4.7     |
| Cervical lymphadenopathy   | 6                           | 14.0    |
| Pneumonia                  | 4                           | 9.3     |
| Diarrhea                   | 26                          | 60.5    |
| Koplik’s spots             | 26                          | 60.5    |

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**Table 2. Patient and Control Groups’ Demographic and Laboratory Data**

| Demographic Characteristics and Some Oxidative Stress Parameters | Patient Group (n = 43) | Control Group (n = 43) | \(P\)   |
|------------------------------------------------------------------|------------------------|------------------------|--------|
| Gender (male/female)                                             | 24/19                  | 31/12                  | >.05*  |
| Age (months)                                                     | 20 (8-120)             | 53 (4-107)             | >.05** |
| TAS (μmol Trolox eqv./L)                                         | 1.37 ± 0.2             | 1.55 ± 0.17            | .031***|
| TOS (μmol HOeqv./L)                                              | 15.91 (6.96-21.8)      | 12.14 (8.58-24.34)     | <.     |
| Native thiol (μmol/L)                                            | 323.1 (199.5-436.4)    | 364.24 (91.09-533.97)  | .005** |
| Total thiol (μmol/L)                                             | 364.3 (224-470.7)      | 395.2 (110.25-562.3)   | .046   |
| Disulfide (μmol/L)                                               | 19.67 ± 5.59           | 14.59 ± 4.57           | .025***|
| % Disulfide/native thiol%                                        | 6.14 (2.92-11.46)      | 4.44 (1.16-10.52)      | <.001**|
| % Disulfide/total thiol%                                         | 5.47 (2.76-9.33)       | 4.08 (1.14-8.69)       | <.001**|
| % Native thiol/total thiol%                                      | 89.06 (81.35-94.48)    | 91.85 (82.62-97.73)    | <.001**|
| OSI                                                               | 1.16 (0.5-1.81)        | 0.78 (0.55-1.58)       | <.001**|

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

*Values are given as median (minimum and maximum values were given in parentheses).

*Values are given as mean ± standard deviation.

*Pearson Chi-Square.

**Mann–Whitney U test.

***Independent-samples’ t-test.
increased compared to the control group. Cemek et al. examined the oxidative stress levels in the patient group were significantly higher than the control group. All these results showed that the plasma malondialdehyde (MDA), the serum carbonyl, and the plasma total sulfhydryl levels in 25 patients with measles were higher in the patient group compared to the control group. Therefore, to provide treatment appropriate to the oxidative stress level of patients with measles, thiol/disulfide homeostasis as biomarkers of oxidative stress can be used in the detection and follow-up of the oxidative stress level.

Availability of Data and Material: The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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