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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

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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.[8-10] They also have a critical role in programmed cell death, detoxification, antioxidant protection, and the regulation of cellular enzymatic activity.[11,12]

Measuring the thiols in serum is an indirect indicator of antioxidant defense.[8,12] 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 pediatrics department of a state university in Turkey. The case group included 43 children with measles and a control group was formed of 43 healthy age and gender-matched children.

Sampling

Sample size was calculated using the Guzelcicek et al[16] method. The results showed that for the disulfide/native thiol to have 0.05 margin of error and 0.95 statistical power, the minimum sample size was 36 subjects to give 1.26 effect size.

The patient group consisted of children, aged 0-10 years, who presented at the outpatient clinic with complaints of maculopapular rash, fever, redness of the eyes, and weakness. In the patients with these findings, the measles immunoglobulin M (IgM) level was measured using the enzyme-linked immunosorbent aasay (ELISA) method. Patients with IgM level > 1.1 RU/mL were included in the study, and 5 patients with IgM levels < 1.1 RU/mL were considered negative. The 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 Erel[22] 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 xylenol 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 H_2O_2 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 H_2O_2 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 H_2O_2 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

test, histogram, scatter plot, and skewness–kurtosis values. Descriptive statistics were shown as mean ± standard deviation (SD), median, and maximum and minimum values for continuous data and as number (n) and percentage (%) for categorical data. The Pearson's chi-squared test was used to compare the categorical data. In comparisons between the patient and the control groups, the independent *t*-test was applied to variables with normal distribution and the Mann–Whitney *U* test to variables that did not show normal distribution. A value of $P < .05$ was accepted as statistically significant.

RESULTS

Descriptive Statistics

Evaluation was made of the patient group of 43 children with measles and the control group of 43 healthy children. Cough and fever were the most common findings in the patient group. Hospitalization for treatment was required by 12 patients with measles (27.9%) (Table 1).

Other Analysis

There was no significant difference between the patient group and the control group in terms of age or gender ($P > .05$) (Table 2). Total antioxidant status was determined to be significantly lower ($P = .03$) in the patient group than in the control group, 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.

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

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

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.

clues about the degree of the free radical-mediated oxidation of proteins which cause damage.[27]

Many studies have shown that oxidative stress and free oxygen radicals play an important role in the pathogenesis of viral infection, especially in hepatitis, influenza, and acquired immune deficiency syndrome.[20,25,30,31] However, there are very few studies in the literature that have examined oxidative stress in patients with measles. Although there is a limited number of studies that have explained oxidative stress in measles by different mechanisms, no study has evaluated thiol/disulfide, TAS, TOS, and OSI parameters altogether. Therefore, in this study, the presence of oxidative stress in patients with measles was examined with respect to the thiol mechanism.

In previous studies in literature, Caksen et al[20] showed that although serum alpha-tocopherol, beta carotene, retinol, ascorbic acid, and glutathione levels were significantly higher in SSPE patients, glutathione level was significantly lower and the oxidative stress levels in the patient group were significantly increased compared to the control group. Cemek et al[18] examined the levels of enzymatic antioxidant parameters (superoxide dismutase, glutathione peroxidase, and catalase) and non-enzymatic antioxidant parameters (ceruloplasmin, albumin, total bilirubin, and uric acid) in children with measles and healthy control subjects. They also found that with the exception of albumin, the levels of the antioxidant parameters increased in the patients with measles compared to the control group. In another 2 studies of patients with measles, oxidative stress levels were seen to be significantly increased in the patient group compared to the control group, which was in line with the findings of the current study.[18,19] Erbay et al[32] examined the plasma malondialdehyde (MDA), the serum carbonyl, and the plasma total sulfhydryl levels in 25 patients with measles and 25 healthy control subjects. From the study results it was reported that the plasma MDA and the serum carbonyl levels were higher in the patient group compared to the control group and the plasma sulfhydryl levels were lower in the patient group than in the control group. All these results showed that the ROS, which is produced by the factors released in measles, causes the formation of disulfide bonds, a decrease in thiol levels, and an increase in OSI values by oxidizing thiol groups in the organism. Therefore, it was thought that the monitoring of TAS, TOS, and OSI parameters could provide important contributions to the determination of the degree of oxidative stress in patients with measles. It would then be possible to develop personalized treatment methods appropriate to the oxidative stress load of each patient. From the results of this study it was concluded that TAS, native thiol, total thiol, and the native thiol/total thiol ratio were significantly lower ($P < .05$) in patients with measles, whereas the TOS, OSI, disulfide, disulfide/native thiol, and disulfide/total thiol levels were significantly higher ($P < .05$). Thus, the results of this study supported the findings of previous literature.

This study had some limitations. Although the patient and control groups were matched as much as possible, exact matching was not possible, as in all case–control studies. In future studies, increasing the number of control groups by selecting from a group that can be generalized to the general population could strengthen the power of the study. In addition, although the measurements were made in the same laboratory environment, it should be considered that there was a temporal difference between the individuals in the measurements. In this study, to minimize this limitation, care was taken to draw blood from the patients during the same period of the disease as far as possible.

CONCLUSION

In conclusion, the detection of oxidative stress in patients with measles is important, and to do this, some laboratory findings can be used in the decision-making process. The results of this study demonstrate that by using thiol/disulfide homeostasis as a biomarker of oxidative stress, a significant increase in the level of oxidative stress in patients with measles was found 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 MaterialThe data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Committee Approval: This study was approved by Ethics committee of Harran University, (Approval No: 19.05.17).

Informed Consent: Verbal and written informed consent was obtained from the patients who agreed to take part in the study.

Peer Review: Externally peer-reviewed.

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