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Original Article

Investigation of thiol-disulfide balance in children with pneumonia

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Abstract

Aim: We aimed to investigate the thiol-disulfide balance, role of oxidative stress in pneumonia and role of measurement of thiol as a new biological marker for the diagnosis of pneumonia in children with pneumonia who admitted to pediatric emergency service and general pediatric polyclinic.

Material and Methods: In our study, patients between 6 months and 4 years of age, who admitted to the Pediatric Emergency Department and Pediatric Policlinic of between January 2017 and February 2018 with a diagnosis of community-acquired pneumonia, were evaluated prospectively. A total of 90 patients were included in the study, including 45 patients and 45 healthy children in the same age group. These patients were examined for age, gender, symptoms and physical examination findings, native thiol, total thiol and disulfide values.

Results: There was no significant difference between the patient and control groups in terms of gender. Statistically significant difference was observed between native thiol, total thiol and disulfide values between patient and control group ($p<0.05$). In the patient group, the levels of native thiol and total thiol were low and disulfide levels were found to be high. There was a significant difference between patient and control group in terms of disulfide / total thiol, disulfide / native thiol and native thiol / total thiol parameters ($p<0.05$). Disulfide / total thiol and disulfide / native thiol parameters were high in the patient group. Nativethiol / total thiol ratio was found to be high in the control group.

Conclusion: In children with pneumonia; native thiol, total thiol, disulfide and their ratios were found to be significantly correlated. It was found that oxidative stress has an important role in the pathogenesis of pneumonia. The use of thiols in the diagnosis of pneumonia may guide clinicians.

Keywords: Child; thiol disulfide balance; pneumonia

INTRODUCTION

Pneumonia is an acute inflammation of the lung parenchyma and is often caused by infectious agents, mainly by bacteria, or non-infectious agents. Pneumonia is a clinical entity which id defined by respiratory findings, radiological findings, fever and physical examination findings of lung parenchymal involvement (1). World Health Organization (WHO) describes pneumonia as a cough or breathing difficulty and an increase in the number of breaths defined by age (2).

Although pneumonia is usually caused by viral and bacterial microorganisms, pneumonia may also develop due to aspiration of food and stomach contents, non-infectious causes such as foreign bodies, hydrocarbons and lipoid substances, hypersensitivity reaction, drug or radiation (3,4).

The etiology of pneumonia is difficult to determine because it is an invasive procedure to obtain a direct culture sample from lung tissue. Culture tests from the upper airways or sputum cannot precisely determine the cause of lower respiratory infection (3). The microorganisms responsible for the etiology in children vary according to age. *Streptococcus pneumonia (pneumococcus) is the most common pneumonia agent in children between 3 weeks and 4 years of age, whereas the most common pneumonia agents in children 5 years and older are Mycoplasma pneumonia and Chylamydophila pneumonia* (5).

Microbiological, serological and molecular tests are performed in the diagnosis and investigation of the etiology of pneumonia (1). Generally, markers such as white blood cell count, C-reactive protein (CRP), absolute neutrophil count, erythrocyte sedimentation rate (ESR) are laboratory tests used to support the diagnosis of pneumonia. However, increased levels of these markers in other inflammatory events decrease their effectiveness in the diagnosis of pneumonia (6). Therefore, various studies have been conducted to find new biomarkers that can be used in determining the diagnosis and severity of pneumonia (7).

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Thiols; sulfur analogs of alcohols containing sulfhydryl (-SH) groups formed by bonding a sulfur and hydrogen atom to a carbon atom. They are also called "mercaptans" because of their mercury binding properties. Thiols are also among the largest and most commonly used antioxidants in plasma. When thiols (RSH) are oxidized by various oxidants, disulfide (RSSR) bonds are formed. The resulting disulfide bonds can be reduced back to thiol groups, thereby maintaining the dynamic thiol / disulfide balance (8).

Disulfides contain adjacent double sulfur atoms. Dynamic thiol / disulfide balance has a role in many mechanisms including antioxidant reactions, detoxification, apoptosis, enzyme activity regulation, transcription, and signal transduction mechanisms (9). There are some studies showing that dynamic thiol / disulfide balance is affected in many diseases. These studies include diabetes (10), cardiovascular diseases (11), rheumatoid arthritis (12), chronic kidney diseases (13), acquired immuno-deficiency syndrome (AIDS) (14), Parkinson's Disease (15), Alzheimer's Disease, Freidreich Ataxis, Multiple Sclerosis, Amyotrophic Lateral Sclerosis (16,17) and some other diseases related studies. The aim of this study was to investigate the relationship between thiol-disulfide levels and the presence and severity of oxidative stress in patients with community-acquired pneumonia and to investigate the utility of thiol disulfide balance as a new biomarker in the diagnosis of pneumonia.

MATERIAL and METHODS

A total of 90 patients including 45 patients between 6 months and 4 years of age who were diagnosed with pneumonia and applied to Harran University Research and Application Hospital Pediatric Emergency Department and Pediatric Outpatient Clinic between January 2017 - February 2018 and 45 healthy children in the same age group were included in this study. The study was carried out with the approval of the Council of Ethics of the Faculty of Medicine of Harran University with the decision no 17 dated 04.01.2018. This study was supported by Harran University Scientific Research Projects Unit with project number 18160. Information form was given to the parents of the children included in the study and informed consent was obtained.

Patients with a history of chronic disease, congenital disease that constitutes a risk factor for pneumonia, a history of continuous drug use, neuromuscular disease, immunodeficiency, and a history of hospitalization in the last 2 weeks were excluded from the study.

Gender, age and vaccination status of the patients and healthy subjects included in the study were questioned. Height, weight and percentiles of all cases were calculated. Diagnosis of community-acquired pneumonia was made due to the presence of fever, respiratory and other physical examination findings, laboratory findings and chest radiograph findings. As the patient group was selected from the patients who were admitted to the pediatric emergency department and outpatient clinic and diagnosed as pneumonia; auxiliary laboratory tests were recorded, blood samples were not taken for auxiliary laboratory tests. The patient group was classified as pneumonia, severe pneumonia and very severe pneumonia according to the clinical classification criteria for pneumonia in Table 1 (1).

Three milliliter venous blood samples for thiol-disulfide levels were taken from all patients and control group. Centrifuged blood samples were stored at -80 ° C. Then all blood samples were studied by ELISA method in Harran University Medical Biochemistry Laboratory.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) and SPSS (Statistical Package for Social Sciences / 17.0 for Windows) program was used for statistical analysis. When evaluating the study data; In addition to descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum), Student t Test was used for comparison of two groups of variables showing normal distribution in the comparison of quantitative data. Spearman's Correlation Analysis was used to evaluate the relationships between variables. Pearson chi-square test was used to compare the qualitative data. ROC analysis was used for cut off estimates of variables. $p<0.05$ was accepted as statistically significant.

Table 1. Clinical Classification in Pneumonia

	Pneumonia	Severe Pneumonia	Very Severe Pneumonia
Consciousness	Normal	There may be a tendency to fall asleep	Lethargy / confusion / Unresponsiveness to painful stimuli
Moaning	No	Possible	Yes
Color	Normal	Pale	Cyanotic
Respiratory Rate	Tachypnoeic	Tachypnoeic	Tachypnoeic – apneic
Breast Withdrawal	No	Yes	Yes
Nutrition	Normal	Reduction in oral intake	No
Dehydration	No	Possible	Yes (shock findings)

RESULTS

A total of 90 patients including 45 patients between 6 months and 4 years of age who were diagnosed with pneumonia and 45 healthy children in the same age group were included in this study. There was no significant difference in terms of gender.

As a result 28.9% (n = 13) of the patients had mild pneumonia, 64.4% (n = 29) had moderate pneumonia and 6.7% (n = 3) had severe pneumonia.

A statistically significant difference was found between the Native Thiol measurements and the values in patient group were lower than the control group (p=0.001). There was a statistically significant difference between the total thiol measurements and the values in patient group were lower than the control group (p=0.001). A statistically significant difference was found between groups in terms of disulfide measurements and the values of patient group were higher than that of control group (p=0.010).

Disulfide / Native Thiol ratios were found to be statistically significant between groups and the patient group rates were higher than the control group (p=0.001).

Disulfide / Total Thiol ratios were found to be statistically significant between groups and the patient group rates were higher than the control group (p=0.001).

Native Thiol / Total Thiol ratios were found to be statistically significant between groups and the patient group rates were lower than the control group (p=0.001) (Table 2).

Table 2. Evaluation of Native Thiol, Total Thiol and Disulfide Measurements by Groups

		Patient group (n=45)	Control group (n=45)	P
Native Thiol (SH)	Min-Max (Median)	285.3-514.6 (413.5)	398.4-730.2 (503.8)	**0.001****
	Mean±SD	416.24±53.41	518.96±76.33	
Total Thiol	Min-Max (Median)	345.6-564.4 (485.7)	420.3-779.7 (560.1)	**0.001****
	Mean±SD	478.82±56.48	569.83±77.08	
Disulfide (SS)	Min-Max (Median)	10.3-71.5 (25.9)	8.2-37.4 (27)	**0.010***
	Mean±SD	31.29±13.05	25.41±7.30	
Disulfide / Native Thiol	Min-Max (Median)	0-0.2 (0.1)	0-0.1 (0)	**0.001****
	Mean±SD	0.08±0.04	0.05±0.02	
Disulfide / Total Thiol	Min-Max (Median)	0-0.1 (0.1)	0-0.1 (0)	**0.001****
	Mean±SD	0.07±0.03	0.04±0.01	
Native Thiol / Total Thiol	Min-Max (Median)	0.7-0.9 (0.9)	0.9-1 (0.9)	**0.001****
	Mean±SD	0.87±0.05	0.91±0.03	

Student t Test *p<0.05 **p<0.01

There was a statistically significant difference between Disulfide / Native Thiol, Disulfide / Total Thiol and Native Thiol / Total Thiol ratios according to the presence of pneumonia (p=0.00; p<0.01, respectively) and disulfide / Native Thiol and Disulfide / Total Thiol ratios were high in the patient group whereas native thiol / total thiol ratio was found to be low. Based on this significance, the cut-off point was considered for Disulfide / Native Thiol, Disulfide / Total Thiol and Native Thiol / Total Thiol ratios. ROC analysis and diagnostic screening tests were used to determine the cut-off point according to the presence of pneumonia (Table 3).

Table 3. Diagnostic Screening Tests and ROC Curve Results for Disulfide / Native Thiol, Disulfide / Total Thiol and Native Thiol / Total Thiol rations

	Diagnostic Scan					ROC Curve		
	Cut off value	Sensitivite (%)	Spesifisite (%)	PPV (%)	NGV (%)	AUC	95% Confidence Interval	P value
SS/SH	**≥0.06**	66.67	75.56	73.17	69.39	**0.755**	0.656-0.853	**0.001****
SS/Total	**≥0.053**	66.67	75.56	73.17	69.39	**0.756**	0.657-0.854	**0.001****
SH/Total	**≤0.89**	64.44	77.78	74.36	68.63	**0.752**	0.653-0.851	**0.001****

PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area

According to the presence of pneumonia, the cut-off point for Disulfide / Native Thiol was determined as 0.06 and above. For cut-off value of 0.06 for disulfide / native thiol; sensitivity was 66.67%; specificity was 75.56%; positive cut-off value was 73.17% and negative cut-off value was 69.39%. The area under the ROC curve was 75.5% and the standard error was 5% (Figure 1).

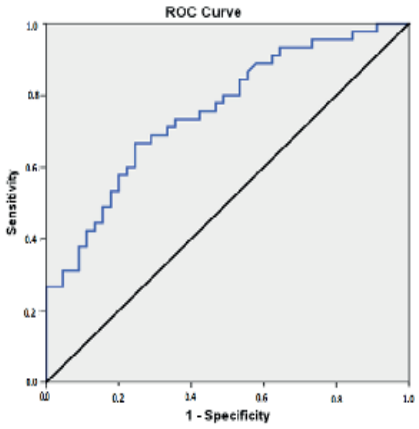

Figure 1. ROC curve for the level of disulfide / native thiol according to the presence of pneumonia

According to the presence of pneumonia, the cut-off point for Disulfide / Total Thiol was determined as 0.053 and above. For cut-off value of 0.053 for disulfide / total thiol; sensitivity was 66.67%; specificity was 75.56%; positive cut-off value was 73.17% and negative cut-off value was 69.39%. The area under the ROC curve was 75.6% and the standard error was 5% (Figure 2).

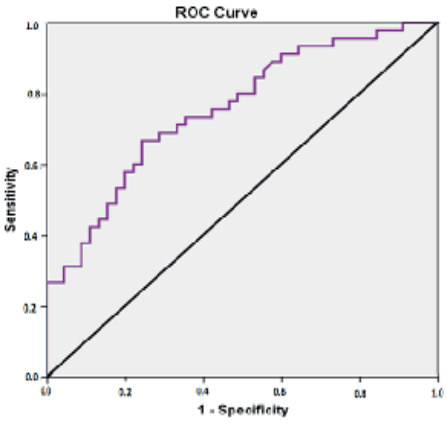

Figure 2. ROC curve for the level of disulfide / total thiol according to the presence of pneumonia

According to the presence of pneumonia, the cut-off point for Native Thiol / Total Thiol was determined as 0.89 and less. For cut-off value of 0.89 for native thiol / total thiol; sensitivity was %64.44; specificity was %77.78; positive cut-off value was %74.36 and negative cut-off value was %68.63. The area under the ROC curve was %75.2 and the standard error was 5.1% (Figure 3).

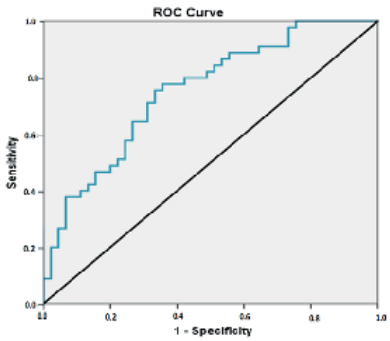

Figure 3. ROC curve for the level of native thiol / total thiol according to the presence of pneumonia

DISCUSSION

Pneumonia is an important cause of morbidity and mortality worldwide in children, especially in developing countries. Therefore, studies on the pathophysiology of pneumonia and studies on new biomarkers that can be used in diagnosis are important. There are some studies showing increased oxidative stress in patients with pneumonia.

The deterioration of oxidative equilibrium as a result of the increase of reactive oxygen species (ROS) and the insufficiency of antioxidant substances and mechanisms that detoxify them is called oxidative stress. Reactive oxygen species, formed as a result of oxidative and antioxidant balance deterioration and increase in oxidative stress, damage intracellular protein, lipid and DNA structures. The presence and degree of oxidative damage can be determined by measuring the products released from these intracellular damaged molecules in body fluids and tissues by various methods. In recent years, studies on determining oxidative damage have gained weight (18).

In a study of Duflo et al. (19), Alveolar and serum oxidative stress was investigated in ventilator-associated pneumonia (VAP). Thiobarbituric acid-reactive substances (TBARS) were collected and analyzed for antioxidant activity in blood and bronchoalveolar lavage samples. This study showed that VAP is associated with early oxidative stress in alveolar fluid and blood.

In a study of Cemek et al. (20), malonaldehyde, reduced glutathione, serum ß-carotene, retinol, vitamin C, vitamin E, catalase, ceruloplasmin, total biluribine, erythrocyte superoxide dismutase and glutathione peroxidase were studied in children with acute pneumonia. In this study, it was shown that oxidative stress increased and enzymic and non-enzymatic antioxidant activities decreased significantly in children with acute pneumonia.

In a study of Aydınoğlu et al. (21), blood plasma total antioxidative status (TAS), plasma oxidative stress index

(OSI) and total oxidative status (TOS) were evaluated in children with parapneumonic effusion. Blood plasma TAS levels were significantly higher in the control group compared to the exudate and transudate groups. In this study, it was shown that increased OSI value in blood plasma of patients with parapneumonic effusion was associated with tissue inflammation and tissue damage.

In a study which was conducted by Bircan et al. (22), the aim was to investigate total antioxidant capacity, malonaldehyde measurement and oxidative stress in patients with community acquired pneumonia and to compare with CRP and pneumonia severity index. In this study, total antioxidant capacity was significantly lower in the patient group than in the control group.

In a study of Parlak et al. (23), Thiolysulfide balance was examined in adult patients hospitalized with the diagnosis of community-acquired pneumonia and in the control group and total thiol and native thiol levels were found to be significantly lower in the patient group compared to the control group. In the same study, there was no significant difference in disulfide levels between the patient and control groups, and disulfide / total thiol ratio was found to be statistically significantly higher in pneumonia cases compared to the control group.

In our study, total thiol and native thiol levels were significantly lower in the patient group. In terms of total thiol and natriethiol evaluation, these two studies investigating pneumonia in different age groups support each other. However, in our study, a significant difference was found in disulfide level and patient group measurements were higher than the control group.

In our study, a statistically significant difference was found between two groups in terms of disulfide / total thiol ratios and ratios of patient group was found to be higher than that of control group.

CONCLUSION

Pneumonia is a serious cause of mortality and morbidity in pediatric patients. Understanding all the mechanisms involved in the pathogenesis of pneumonia with the support of new studies can contribute to the diagnosis and treatment of this disease. Within this scope, we investigated thiol-disulfide balance in children with pneumonia in our study. We found that the values of native thiol and total thiol decreased significantly, disulfide values increased and disulfide / native thiol, disulfide / total thiol ratios increased in children with pneumonia. All these data show the presence of oxidative stress and changing antioxidant / oxidant balance in the pathogenesis of pneumonia. In this study, we determined the impaired thiol-disulfide balance in the presence of pneumonia but further studies investigating oxidative stress in disease pathogenesis are needed.

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

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Ethical approval: The study was carried out with the approval of the Council of Ethics of the Faculty of Medicine of Harran University with the decision no 17 dated 04.01.2018.

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