The Effects of Asthma on the Stress Oxidative, Inflammation, and Endothelial Dysfunction Characteristics in Children with Severe Community-Acquired Pneumonia

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Abstract

Background: Pulmonary vascular endothelial activation, inflammation, and stress oxidative have been implicated in adverse clinical outcomes of community-acquired pneumonia (CAP). Chronic lung problems such as asthma may affect the consequences of pneumonia.The present study aimed to assess the effects of asthma on the oxidative stress, inflammation, and endothelial dysfunction biomarkers in children pneumonia. Methods: This cross-sectional study was performed at Amir Kabir Hospital affiliated to Arak University of Medical Sciences, Arak, Iran. Participants were 25 children with severe CAP and asthma (group I), 25 children with severe CAP (group II), and 25 healthy children (group III) with 2 to 6 years of age. Fasting blood samples were taken to the assay of serum malondialdehyde (MDA), total antioxidant capacity (TAC), tumor necrosis factor-alpha (TNF-α), soluble vascular cell adhesion molecule-1 (sVCAM-1), and Plasminogen activator inhibitor-1 (PAI-1). Results: We observed a significant reduction in TAC in groups I and II compared with group III. This reduction was significantly higher in group I than in group II. Also, we observed a significant increase in MDA and TNF-α in groups I and II compared with group III. The increase in MDA was significantly higher in group I than in group II. VCAM-1 and PAI-1 as endothelial dysfunction biomarkers increased significantly in group I compared with groups II and III. Also, VCAM-1 and PAI-1 increased significantly in group II compared with groups III. Conclusions: Asthma can exacerbate the consequences of pneumonia in children by increasing oxidative stress, inflammation, and endothelial dysfunction.

Introduction:

Pneumonia is an infection of the lungs caused by bacteria, viruses, fungi, and parasites that impose significant costs for the health care system and exhibit the most common reason for the death of infectious origin[1]. In this disease, polymorphonuclear neutrophils and macrophages fight with microorganisms by using reactive oxygen species (ROSs) and lysosomal enzymes[2]. As a consequence of pulmonary defense mechanism in inflammatory diseases such as pneumonia and asthma, Oxidative stress (OS) at the systemic level may have a central role with adverse clinical outcomes of these diseases, such as the endothelial dysfunction (ED), exacerbation of inflammation, and shortness of breath, and ultimately acute respiratory distress syndrome (ARDS), and death [3-5].

ED causes pulmonary edema due to an increase in endothelial permeability. The activated endothelium mediates leukocyte binding to express the adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) that mediate leukocyte binding. Upon leukocyte binding, these adhesion molecules activate endothelial cell signal transduction and then alter endothelial cell shape for the opening of passageways, through which leukocytes can migrate [6-7].

Characterized by chronic inflammation in the airway wall, asthma is the most common chronic respiratory disease in children, which is prevalent in developing countries. Although it cannot be considered a direct cause

of pneumonia, children with asthma are more prone to develop pneumonia due to previous lung damage. As a result, a child with asthma may have more severe symptoms and complications from pneumonia. Asthma may exacerbate the clinical consequences of pneumonia, such as ED[8-11].

We are not aware of any studies on assessing the changes in OS, inflammation, and ED biomarkers in children with asthma and pneumonia together compared with children with pneumonia only. Therefore, the current study assessed the alterations in OS, TNF-α, and ED biomarkers in children with asthma and pneumonia, children with pneumonia only, and healthy children.

Materials and methods

Study design and participants

A cross -sectional study was conducted at the Pediatric Clinic of Amirkabir Hospital in Arak, Iran, from January 2019 to September 2019. To estimate the sample size, we considered type 1 (α) and type 2 errors (β) of 0.05 and 0.20 (power=80%), respectively, and serum MDA level as a key variable. Based on a previous study[12], SD (σ1) of control MDA was 0.1 μmol/L, SD (σ2) of case MDA was 0.07 μmol/L, and the difference in mean (d) of insulin levels was 0.15 μmol/L. We reached the sample size of 9 participants for each group. The sample size of our study consisted of 25 children diagnosed with severe community-acquired pneumonia (sCAP), 25 patients diagnosed with asthma and sCAP, and 25 healthy children.

Pneumonia was defined as an acute pulmonary infiltrate evident on chest radiography with symptoms and signs of a lower respiratory tract infection: fever, cough, and purulent sputum. Pneumonia was confirmed with physical exams, microbiologic culture data, and Chest x-ray. CAP in children was defined as a lower respiratory tract infection in a child who has not resided in a hospital or health care facility in the preceding 14 days. CAP in children is one of the most common acute infections that require going to the hospital. Children with sCAP, due to respiratory distress, are not able to eat, drink, and alert. They also have undesirable hydration status and oxygenation status[13-14].

The asthma of children was confirmed by a physician via the symptoms of recurrent coughing, wheezing, and chest tightness.

Exclusion criteria included children with severely smoking parents and severe or multiple systemic diseases.

Ethical and Safety Considerations

The present study was ethically approved by the Committee on Human Research, Publication and Ethics (CHRPE) at Arak University of Medical Sciences, Arak, Iran (IR.ARAKMU.REC.1397.3001). All parents completed a written consent form and signed/thumb-printed. Then the study was explained to them in their language.

Biochemical assessments:

Blood samples of all the subjects were taken, and aliquot samples of serums were saved after centrifugation (20 min, 3000 rpm) at -80°C.

According to the method of Benzie & Strain in 1996[15], TAC was analyzed using fluorescence recovery after photobleaching (FRAP*)* assay, which depends on the capacity of serum to reduce Fe^{3+} to Fe^{2+}.

Serum MDA levels were determined by the thiobarbituric acid reactive substances test (TBARS) spectrophotometric test, as described by Santos in 1980 [16].

PAI-1 was measured as an indicator of ED by ELIZA kit (Germany, ZellBio, ZB-11159C-H9648). Also, VCAM-1, as another indicator of ED, was measured by the ELIZA kit (France, Diaclone SAS, 25020).

Serum TNF-α was measured through the ELISA method according to the manufacture's instruction (Biovendor, Germany, Cat[?] RAF128R).

Statistical analysis

The Kolmogorov–Smirnov test was employed to assay the normal distribution of variables. The one-way ANOVA and Kruskal-Wallis test were employed to compare Anthropometric and Biochemical factors between groups. Post Hoc and Mann-Whitney tests were utilized to compare subgroups (I, II, III). All statistical analyses were performed using SPSS version 17 (SPSS, Chicago, IL, USA).

Result

This study was conducted from January 2019 to September 2019. It consisted of 25 children with pneumonia and asthma (group I), 25 children with pneumonia (group II), and 25 healthy children (group III) with 2 to 6 years of age.

Table 1 presents the children's anthropometric variables.

Results showed a significant reduction in TAC in groups I and II (0.997±0.22 and 1.23±0.21mmol/l, respectively) compared with group III (1.46±0.19 mmol/l), which was higher in group I than in group II. Also, a major increase was observed in MDA and TNF-α in groups I (2.57±0.40μmol/l, 6.94±1.61mmol/l, respectively) and II (2.11±0.26 μmol/l, 5.54±1.84mmol/l, respectively) compared with group III (1.89±0.27

μmol/l, 3.42±1.32mmol/l, respectively), which was significantly higher in group I than in group II (Table2). VCAM-1 and PAI- 1 as ED biomarkers increased significantly in group I (1.5.±0.62 mmol/l and 10.52±3.2 AU/ml, respectively) compared with groups II (1.06±0.53 mmol/l and 8.23±3.4 AU/ml, respectively) and III (0.6± 0.35 mmol/l and 2.39±0.83 AU/ml, respectively). VCAM-1 and PAI- 1 increased significantly in group II compared with groups III (Table2).

Discussion:

In this study, we observed that in children with sCAP, biomarkers of OS, inflammation, and ED were significantly higher than healthy children, and it is also higher in asthmatic children with pneumonia than in non-asthmatic children. This is probably because asthma may exacerbate OS and inflammation in children with pneumonia.

Studies have shown the interaction between pneumonia and cardiovascular diseases (CVDs). According to the cohort study of Yeh et al. 2019, patients with CVDs had a higher risk of CAP, and conversely, CVDs risk was intensified with CAP. In recent years, CVDs were considered as an outcome of patients admitted to hospital with pneumonia infection[17]. After recovery of CAP in addition to the period of the acute infection, there is still the risk of acute cardiovascular events due to systematic inflammation [18].

The initial stage of molecular and cellular stages leading to CVDs is ED (19-20). OS and inflammation are the two main causes of its creation[21-22].

Studies indicate the underlying respiratory diseases such as asthma may be effective in the severity of pneumonia injuries. Asthma, whose main feature is chronic inflammation in the airway wall, is the most common chronic respiratory disease in children, especially in developing countries [8].

In this study, TNF-α was significantly higher in children with pneumonia and asthma than pneumonia and healthy children. Studies indicate the inflammatory process associated with ED exacerbates the severity of the consequences of CAP [23]. Also, recent evidence suggests a critical role for pneumonia infection in the pathogenesis of atherosclerosis by exacerbating OS, inflammation, and ED. Increasing the pro-inflammatory cytokine TNF-α as a consequence of pneumonia induce ED by various mechanisms, such as increasing the endothelial permeability and reducing the endothelium-dependent relaxation. It increased vascular endothelial growth factor (VEGF) as the endothelial permeability mediator and diminishing the half-life of mRNA encoding for endothelial nitric oxide synthase and decreasing nitric oxide production[24, 25].

In this study, VCAM-1 and PAI-I as two biomarkers of ED were significantly higher in children with pneumonia and asthma than the children with pneumonia only. Also, they were significantly more in children with pneumonia than healthy children. OS and inflammation are closely linked with each other. Inflammatory mediators lead to OS, and reciprocally, OS increases the production of inflammatory mediators with the

activation of NF-kB and AP-1[26]. NF-ϰB and AP-1 are involved in the activation of pro-inflammatory molecules, such as vascular cell adhesion molecule one (VCAM-1) and PAI-I [27].

In 2015, Lin et al. indicated that TNF-α-induced VCAM-1 expression in human cardiac fibroblasts was mediated by the activation of NF-ϰB by c-Src-mediated transactivation of the EGF receptor (EGFR)/PI3K/Akt cascade [28]. ROSs regulate several cells signaling pathways, such as expression of VCAM-1, resulting in the release of inflammatory mediators [29].

Zhang et al. (2018) reported an increase in MDA and TNF-α and a decrease TAC in CAP (30). Pikuza et al. (2012) reported an evaluation of the content of MDA as the lipid peroxidation indicator with decreasing of antioxidant activity in CAP patients[31]. Majewska et al. (2004) ascertained OS development in the lungs at CAP patients[32]. Muravlyova et al. (2016) showed that sCAP patients have more levels of oxidative proteins and MDA in erythrocytes than moderate CAP and healthy volunteers [33].

ROSs concentration and time of exposure are two determining factors in the effects of OS in the airway as well as in other organs. Due to damage in biomolecules and inducing intracellular signaling pathways by ROSs, more concentration and longer exposure of ROSs can lead to cell death by apoptosis [34]. Accordingly, studies show that the attenuation of OS alleviates the organ damage.

Zhang et al. in 2018 demonstrated the treatment of CAP patients with N-acetylcysteine (NAC) reduces MDA and increases TAC compared with those in the non-NAC group [30]. In asthma as a chronic inflammatory airway disease, OS exacerbates airway inflammation by inducing various pro-inflammatory moderators, boosting bronchial hyperresponsiveness, exciting bronchospasm, and increasing mucin secretion [35].

Conclusions: Significant changes in OS, inflammation, and ED biomarkers occur in asthma children with pneumonia compared with pneumonia children without asthma and healthy children. Our findings amplify the growing evidence supporting the concept that endothelial activation, inflammation, and OS play an important mechanistic role of effects asthma in the pathogenesis of pneumonia. Treatment with antioxidants and anti- VCAM-1 pharmacological agents may help reduce outcomes in these children.

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Authors' contributions

FI and AAS designed the experiment and supervised the project. FI, AAS, and HK performed the experiments and conducted the lab work. PM conducted the statistical analysis. FI and AAS wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

All data used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Committee on Human Research, Publication and Ethics at Arak University of Medical Sciences, Arak, Iran (IR.ARAKMU.REC.1398.3001). Satisfaction of parents of children was obtained for using the serum samples of their children for measuring biomarkers in this study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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