Effect of Vitamin C Administration on Leukocyte Vitamin C Level and Severity of Bronchial Asthma

Ebrahim Nadi[1], Farnaz Tavakoli[1], Fatemeh Zeraati[2], Mohamad Taghi Goodarzi[3], and Seyed Hamid Hashemi[4]

[1] Department of Internal Medicine, Shahid Beheshti Hospital, Hamadan University of Medical Sciences, Hamadan, Iran

[2] Department of Pharmacology, Hamadan University of Medical Sciences, Hamadan, Iran

[3] Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

[4] Department of Infectious Disease, Hamadan University of Medical Sciences, Hamadan, Iran

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Abstract- Oxidative stress mediated by reactive oxygen species is known to contribute to the inflammatory process of bronchial asthma. Reactive oxygen species are released into the bronchial tree by activated inflammatory cells. In this study, we aimed to determine the effect of vitamin C administration on leukocyte vitamin C level as well as severity of asthma. In this double blind clinical trial study we evaluated 60 patients with chronic stable asthma. The patients were divided into two groups (A and B) including 30 patients in each group. Patients in these groups were matched according to their age, weight, height, gender, BMI and drug consumption. In addition to standard asthma treatment (according to stepwise therapy in 4th step of bronchial asthma) in which the patients were controlled appropriately, group A received 1000 mg vitamin C daily and group B received placebo. At the baseline and after one month treatment, non-fasting blood samples were drawn for laboratory evaluations. Asthmatic patient's clinical condition was evaluated through standard pulmonary function test (PFT). The mean (±SD) leukocyte vitamin C level in group A at the baseline and after one month treatment with 1000 mg/day vitamin C, were 0.0903 (±0.0787) µg/10^8 leukocytes and 0.1400 (±0.0953) µg/10^8 leukocytes respectively (P<0.05). The mean (±SD) leukocyte vitamin C level in group B at the baseline and after one month administration of placebo, were 0.0867 (±0.0629) µg/10^8 leukocytes and 0.0805(±0.0736) µg/10^8 leukocytes respectively. The leukocyte vitamin C level in group A was higher than those of group B after one month treatment with vitamin C and placebo and the difference was statistically significant (P<0.05). Comparing PFT (FEV_1, FVC and FEV_1/FVC) in group B during the study period showed a significant increase in FEV_1 (P<0.05), while the other two parameters remained unchanged. In group A, who received 1000 mg/day vitamin C, none of the spirometry parameters changed after one month treatment, indicating no effect of vitamin C treatment in the spirometry parameters.

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Introduction

Asthma has been a major focus for clinicians in recent years because both the incidence and mortality appear to be increasing, especially within certain ethnic or geographical groups (1-3). No matter what is the cause, the incidence of asthma has appeared to increase and worsen over the recent years, even if it seems to be more stable recently (4,5). Besides, asthma has become increasingly more difficult to treat. Several studies indicate that the incidence of status asthmaticus patients seen in emergency rooms has increased and the mortality of asthma is higher. The mortality appears to be increasing despite many newer drugs using for asthma (6).

Many studies have evaluated the association between antioxidant vitamins (A, C and E) and asthma (1-10), but there seems no common agreement on this controversial topic. Oxidative stress process is the major theory that has tried to explain the effect of antioxidant factors in asthma. Oxidant stress affects inflammatory status, the level of tissue distraction in the respiratory and immune system. Dietary, genetic and environmental factors, which decrease the cellular reducing capacity,

Corresponding Author: Fatemeh Zeraati
Department of Pharmacology, Hamadan University of Medical Sciences, Hamadan, Iran
Tel: +98 811 8380462, 918 3122063, E-mail: zeraati@umsha.ac.ir

will raise tissue vulnerability to oxidant stress and are likely to increase the risk of asthma (1-4).

Many studies have shown that cells involved in the asthmatic inflammatory process, have a major capacity for producing reactive oxygen species (ROS). Activating eosinophils, neutrophils, monocytes and macrophages can generate superoxides (O_2^-) via the membrane associated with NADPH-dependent complex. Subsequently, dismutation of O_2^- could result in hydrogen peroxide (H_2O_2). O_2^- and H_2O_2, which are moderate oxidants, are dangerous in the formation process of potent cytotoxic free radicals in biological systems through their interaction with other molecules. This pathway is involved in asthmatic inflammation. In fact, the concentration of nitric oxide (NO) is increased in airways of asthmatic subjects (8). In addition to recruited inflammatory cells, epithelial airway cells are also potential sources of ROS production (9). Several asthma mediators including lipid mediators, chemokines, adhesion molecules and eosinophil granule proteins are potential promoters of ROS production. In addition to endogenous sources, environmental factors linked to asthma such as air pollutants, are important (10-12). A rise in the ROS production is problematic because oxidation of proteins, DNA and lipids may lead to direct tissue damage and incite a variety of cellular responses through the generation of secondary reactive species (13).

Many epidemiological studies have reported that low total vitamin A, C and E intake is associated with deficits in spirometric parameters, and some studies have shown lower lung function levels in patients with an inadequate dietary antioxidant vitamin intake (13). The data is stronger for vitamin C, one of the key antioxidant vitamins.

Vitamin C is an important water-soluble substance present in two biologically active forms: ascorbic acid and its oxidized derivative, dehydro-ascorbic acid. Vitamin C can act as a hydrogen donor to reverse oxidation and therefore may be termed as an antioxidant that reacts with free radicals and deactivates them before they damage proteins or lipids (10-13). Oxygen metabolites can have a direct or indirect role in the modulation of airway inflammation. Many researchers have suggested that superoxide dismutase and free radical scavengers in blood are significantly lower in asthma and showed a correlation between asthmatic severity and ROS products in asthmatic subjects (14-20). Epidemiological studies suggest that higher intake of dietary vitamin C may be associated with a reduced risk of asthma (21-27).

Therefore, there are evidence proving relationship between antioxidant vitamins and asthma. Many studies showed that these vitamins have a preventive effect and most of them have compared the level of these antioxidants between asthmatic patients and healthy groups. In this double blind clinical trial study, we tried to evaluate the role of vitamin C in the treatment of severe asthma.

Materials and Methods

A double blind clinical trial study was designed to assess the effect of vitamin C administration on leukocyte vitamin C level as well as pulmonary function tests in patients suffering from severe asthma who had referred to Ekbatan hospital in Hamadan (Iran), between October 2009 and January 2010. Among the asthmatic patients attending the hospital, those with severe asthma [chronic stable asthma step 4, according to 2004 Global strategy for asthma management and prevention guideline (18)] were randomly divided in two groups A and B (including 30 patients in each group). Both patients and physician were unaware of details of patient's distributions. The inclusion criteria for all cases were bronchial asthma, where the diagnosis was established through demonstrating reversible airway obstruction. The participants were asked to fill in a questionnaire for identifying their demographic characteristics such as age, sex, asthma history, past medical history and details related to current asthma exacerbation, nocturnal and diurnal symptoms. In order to identify the severity of asthma, a trained observer assessed airway reversibility, peak flowmetry and spirometry in the asthmatic patients. At least three acceptable maneuvers, considering American College of Chest Physicians standards, were required, with at least two reproducible forced expiratory volumes in 1 second (FEV_1) and forced vital capacity (FVC) maneuvers within 5% of best required for each test (28). The airway responsiveness was performed in a standardized fashion and the airway reversibility was evaluated by spirometry before and 15 minutes after inhalation of two puffs of a β-adrenergic agonist (200 micrograms albuterol) as metered dose inhaler and equal or more than 12% increase in FEV1 meant diagnostic for asthma (29). Peak expiratory flow (PEF) was also utilized to assess acute asthma severity and was expressed as percentage of the value according to age, sex, race and height. Changes in PEF were expressed as the relative change in percentage of predicted value. According to National Asthma Education and

Prevention program method, asthmatic patients were categorized in step 4 (18).

Patients in two groups A and B were matched according to their age, weight, height, gender, BMI and drug consumption and were informed about the aims and possible benefits that could be derived from the study. Informed written consent was obtained from each subject. The study protocol was approved by the local ethical committee. Smokers and patients with other chronic diseases were excluded from the study.

In addition to standard asthma treatment (according to stepwise therapy in 4^{th} step of bronchial asthma) in both group, in which patients were controlled appropriately, group A received 1000 mg vitamin C daily and group B received placebo (18).

These patients were under observation by physician and followed up through periodic visits in the hospital for one month. At the baseline and after a month, the subjects recalled for laboratory evaluations. Non-fasting blood samples (10 cm^3) were drawn from the antecubital space of the forearm into large tubes containing a separator solution. The plasma was separated by centrifugation. In order to isolate the leukocytes, after precipitation of the red blood cells, the supernatant was centrifuged at 800–1000 g for 5 minute in large tubes and the precipitate was washed with distilled water and saline solution. Leukocyte samples were deproteinized with a trichloroacetic acid solution for vitamin C assays. Vitamin C in the protein-free supernatant was determined by a colorimetric method using 2,4-dinitrophenylhydrazine (30). The cut-off value for deficient leukocyte vitamin C level was < 20 $\mu g/10^8$ leukocytes, according to NHANES II (20). Asthma outcome was evaluated with standard pulmonary function test (PFT). Statistical analysis was carried out using SPSS for Windows, version 13. Paired t-test and independent t-test were utilized to compare age, weight, height, BMI and leukocyte vitamin C within and between two groups.

Results

The baseline characteristics of the two groups confirmed that they were well matched (Table 1). The mean (± SD) age of patients in group A was 48.38 (± 9.03) years and in group B was 40.53 (± 10.48). There were no significant differences in age, height and BMI between two groups (Table 1).

The mean (±SD) leukocyte vitamin C level in group A at the baseline and after one month treatment with 1 gram vitamin C daily, were 0.0903 (±0.0787) $\mu g/10^8$ leukocytes and 0.1400 (±0.0953) $\mu g/10^8$ leukocytes respectively. There was a significant rise in leukocyte vitamin C level after treatment in group A ($P<0.05$). The mean (±SD) leukocyte vitamin C levels in group B, at the baseline and after one month administration of placebo were 0.0867 (±0.0629) $\mu g/10^8$ leukocytes and 0.0805 (±0.0736) $\mu g/10^8$ leukocytes respectively. There seemed no important difference in leukocyte vitamin C level after 1 month treatment with placebo in group B ($P>0.05$) and there was no significant diversity in the baseline leukocyte vitamin C level between two groups A and B ($P>0.05$), but the leukocyte vitamin C level after one month treatment with 1000 mg/day vitamin C in group A was higher than that in group B (receiving placebo), and the difference was statistically significant ($P<0.05$).

Comparing spirometry parameters (FEV_1, FVC and FEV_1/FVC) in group B during the study period showed a significant increase in FEV_1 ($P<0.05$), while the other two parameters remained unchanged (Table 2). In group A, who received 1000 mg/day vitamin C, none of the spirometry parameters changed after one month treatment (Table 3), indicating no effect of vitamin C treatment on the spirometry parameters.

Table 1. The baseline characteristics of the two groups treated with vitamin C (A) and placebo (B).

Parameters	Group A		Group B		*P*-value*
	Mean	Std. deviation	Mean	Std. deviation	
Age (year)	48.38	9.03	40.53	10.48	0.576
Weight (kg)	63.03	8.81	67.61	12.32	0.048*
Height (cm)	159.80	8.16	163.15	8.06	0.993
BMI (Kg/M×M)	24.92	3.95	25.26	4.50	0.734

* paired t-test

Table 2. The spirometry parameters in patients who have received placebo (groupB) before and after treatment.

Spirometry parameters	Before treatment	After treatment	*P*-value*
FEV_1 (Lit.)	1.63±0.68	1.82±0.78	0.044*
FVC (Lit.)	2.25±0.78	2.33±0.73	0.220
FEV_1/FVC (%)	71.90±15.34	76.07±13.20	0.115

* paired t-test

Table 3. The spirometry parameters in patients who have received one gram vitamin C daily for 1 month (group A) before and after treatment.

Spirometry parameters	Before treatment	After treatment	*P*-value*
FEV_1 (Lit.)	1.40 ± 0.56	1.44 ± 0.59	0.65
FVC (Lit.)	2.14 ± 0.65	2.16 ± 0.54	0.87
FEV_1/FVC (%)	68.38 ± 17.24	65.92 ± 18.68	0.36

* paired t-test

Discussion

It has been suggested that antioxidants might have an etiologic role in bronchial asthma and, if so, this could lead to the development of new therapeutic strategies. The data is stronger for vitamin C, one of the key antioxidant vitamins (31). Our study showed that in spite of 1000 mg/day administration of vitamin C for one month and higher level of this vitamin in patient's leukocyte in group A, the pulmonary function test parameters (FEV_1 and FEV_1/FVC) did not increase and the patients in group A had lower pulmonary function in comparison to patients in group B.

Leukocyte vitamin C status seemed to be more affected by asthmatic status than plasma vitamin C, whereas plasma vitamin C was more influenced by dietary intake of the vitamin. Some studies report that plasma vitamin C concentrations are more indicative of recent vitamin C intake than of body stores. Indeed, plasma vitamin C has a linear relationship with intake of vitamin C. Leukocyte vitamin C concentration is more reflective of tissue stores of vitamin C (32-34), so leukocyte vitamin C would be a more sensitive indicator of asthma duration. Kelly *et al*. reported a decrease in vitamin C and tocopherol in mild asthmatic patients and concluded that reliance on plasma measurement alone is not a sufficient indicator of vitamin C status and highlights the fact that the nature of the relation between plasma and vitamin C pools is unknown (17).

Vallance *et al.* reported that subjects who had undergone infarction, infection and surgery, had leukocytosis that led to decrease in leukocyte vitamin C concentration (35). In the present study, none of the patients had any chronic disease (except asthma) or history of recent surgery, so there was no leukocytosis due to other diseases. Many studies demonstrate that a low dietary intake of vitamin C seems to increase the risk of asthma (1-5). Other studies show that vitamin C reduces the number and severity of attacks in patients suffering asthma and reduces the severity of the bronchial responses to exercise (36). In the research for a possible relationship between vitamin C and asthmatic symptoms, both plasma and leukocyte levels of vitamin C have been studied (9-13). They mentioned that, asthmatic subjects have low plasma and leukocyte concentrations of vitamin C but the relationship between vitamin C levels and duration of asthma and effect on treatment has not been demonstrated. On the other hand, the epidemiological evidence about the role of dietary vitamin C in asthma is controversial (10,12).

Romieu *et al.* (22) studied the effects of vitamin C supplementation in a double-blind trial with placebo, using 158 Mexican asthmatic children randomized to receive 250 mg/daily for 19 months and exposed to acute effects of ozone, nitrogen dioxide and particulates. The authors observed no association between the acute effects of exposition to ozone and the lung functions in the supplementation group, whereas they observed notable lowering of lung functions between two groups for FEF25-75 and peak expiratory flow (PEF). They concluded that the supplementation with anti-oxidants, might modulate the impact of ozone exposure on small airways in children suffering from moderate-to-severe asthma. Similar results were found by Trenga *et al.* (23) in another double-blind crossover study in which the authors evaluated the effects of dietary antioxidant vitamins (C and E) on ozone-induced bronchial hyper-responsiveness (BHR), suggesting that supplementation benefits asthmatic adults exposed to air pollutants.

The NAHSIT examined the association between nutrient intake, physician-diagnosed asthma and allergic rhinitis in 1166 adolescents (13–17 years). The authors found a marginal significance between vitamin C intake in the lowest quartile and an elevated risk for asthma (24). Kongerud *et al.* (25) found that induced sputum decreases levels of ascorbic acid in airways of 16 mild-asthmatic subjects, compared with 18 healthy controls. On the contrary, many studies do not confirm the relationship between asthma and vitamin C (26). In a recent randomized placebo-controlled trial using 300 asthmatic patients (18-60 years), Fogarty *et al.*, (37) examined the association between vitamin C supplementation (1000 mg/day for 16 weeks) and the improvement of clinical control asthma (FEV1, FVC, BHR, mean morning and evening peak flow, symptoms

score, and bronchodilators use). The results demonstrated that a regular vitamin C dietary supplementation did not add any clinical benefits respect to current standard therapy of asthma in primary care patients evaluated. Importantly, Kalayci *et al.* (27) did not observe any correlation between antioxidant vitamins and lipid peroxidation products in 14 asthmatic children. In this study, the antioxidant vitamins were decreased in sera of asthmatic patients even during the asymptomatic period of disease, and this decrease was not dependent on increased oxidative stress as reflected by lipid peroxidation products. On the basis of the studies investigating the role of antioxidant substances in asthma, there is currently no consistent conclusion, because the majority of studies have been short, with different dosage supplementation, and assessed the immediate effects of antioxidants vitamins. In our study, we evaluated the effect of one of the antioxidants (vitamin C) in severe asthma (step 4). Stage of disease is important to response to any medications and it may be one of the reasons that vitamin C had no effect in our study. We can conclude, in spite of higher level of leukocyte vitamin C level after one month treatment, no significant changes occurred in spirometry parameters. Some potential limitations of our study were the small sample size and short time administration of vitamin C.

Long-term supplementation controlled-studies with placebo are needed to clarify the role and effects of anti-oxidants in the asthmatic inflammatory process.

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