Serum levels of antioxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma

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SUMMARY: Kalaycı Ö, Besler T, Kılınç K, Şekerel BE, Saraçlar Y. Serum levels of antioxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma. Turk J Pediatr 2000; 42: 17-21.

We determined serum levels of alpha tocopherol, beta carotene, and ascorbic acid and lipid peroxidation products (thiobarbituric acid reactive substances-TBARS) in 14 children during an asthma attack and remission. Twelve healthy children served as controls. All antioxidant vitamins were significantly lower in asthmatics at remission compared to controls. Comparison of attack and remission periods in asthmatic patients failed to reveal any difference except in beta carotene ($p = 0.03$). The levels of all three vitamins correlated very significantly with each other ($r = 0.89$-0.95). TBARS levels were significantly higher at asthma attack compared to remission ($p = 0.001$). No correlation was observed between the antioxidant vitamins and lipid peroxidation products. This study shows that antioxidant vitamins are decreased in sera of asthmatic patients even during the asymptomatic periods of the disease, and that this decrease is not totally dependent on the increased oxidative stress as reflected by lipid peroxidation products. The role of antioxidant vitamins in prevention and/or treatment of asthma remains to be determined.

Key words: alpha tocopherol, ascorbic acid, beta carotene, bronchial asthma, thiobarbituric acid reactive substances.

A variety of mediators are released by the inflammatory cells that reside within the lung and cause the tissue destruction and inflammation that are characteristic of asthma. Among these mediators are reactive oxygen (ROS) produced by eosinophils, neutrophils, and macrophages. The role that is played by ROS in asthma has been demonstrated by in vitro studies[1-13]. The data from in vivo systems, however, are scarce and conflicting. In the in vivo studies, as the direct measurement of ROS in biologic systems is difficult due to their high reactivity or rapid clearance measurement of lipid peroxidation products has been used as a sensitive indicator of ROS activity[14]. In this context, Owen et al.[15] and Rahman et al.[16] have shown increased lipid peroxidation in asthmatics, whereas the data obtained by Chilvers et al.[17] failed to show the presence of circulating products of ROS in asthma.

Several antioxidant defenses are normally present in pulmonary tissue including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, vitamin C, vitamin E, and β carotene[18]. It has recently been demonstrated that in addition to enhanced lipid peroxidation, vitamin C and vitamin E are decreased in the BAL fluid of guinea pigs following an asthmatic response[18]. The data regarding the relationship between antioxidant vitamins and asthma is limited. Recently, Dow et al.[19], using a food frequency questionnaire, showed that vitamin E may influence lung function in the elderly, whereas vitamin C did not have this effect. The studies with vitamin C have in general failed to produce consistent results[20]. The aim of this study was to investigate the possible changes in antioxidant vitamin levels and their relationship to the oxidant stress produced by asthma. For this purpose, we measured the serum levels of thiobarbituric acid reactive substances (TBAR) and of antioxidant vitamins, ascorbic acid (vitamin C), α tocopherol (vitamin E) and β carotene (provitamin A) in a group of asthmatic children

and compared them to the levels obtained from healthy controls. Levels were determined during a period of hypoxia (acute asthma attack) and 30 days after discontinuation of all medical treatment.

Material and Methods

Patients and Controls

Fourteen children, aged 13-15 years, who presented with a moderate to severe acute asthma attack[21] to the emergency department of Hacettepe University, İhsan Doğramacı Children's Hospital were included in this study. The asthma attack was due to allergens in 11 and to nonspecific airway irritants in three patients. None of the children had malnutrition or growth retardation.

Twelve healthy age-matched children without any systemic or atopic diseases, who also had normal physical examination findings, served as the control group.

Design

Blood (15-20 ml) was drawn from the radial artery of each patient upon presentation with an acute asthma attack. From this sample blood-gas determination was done and serum was separated and stored at -20 °C until analysis. Pulmonary functions were also measured (2130 PFT system. Sensor Medics, CA, USA). All patients were treated according to a standard protocol with oral corticosteroids and inhaled salbutamol. Upon complete recovery from the acute excarbation, both medications were discontinued and patients were called for a follow-up visit in 30 days. At the follow-up visit, in addition to measuring pulmonary functions, all blood tests that were done at study entry were repeated. One day later, in order to determine their atopic status, all patients were skin tested with common aeroallergens and food allergens.

Serum lipid peroxide levels were determined by measuring thiobarbituric acid (TBA) reactivity as described by Wade and van Rij[22].

Determination of Vitamins

Serum Vitamin E concentration was measured using the method described by Desai[23]. The intra-and inter-assay of coefficient variation for the measurement were 2.1 and 3.4 percent, respectively. The colorimetric method was employed for asay of beta carotene[24,25]. Intra- and inter-assays of coefficient variation in beta carotene measurements were calculated as 3.8, and 1.8 percent, respectively. Analysis of vitamin C was conducted using the colorimetric method using 2,6-dichlorophenol-indophenol[26].The intra-and inter-assay variations for the vitamin C assay in the study were 2.7 and 4.4 percent, respectively.

Statistical Analysis

All statistical analyses were done using SPSS 6.0 statistical program. For comparisons between the acute attack and remission periods of asthma patients, Wilcoxon paired samples test was used, and comparison of asthma patients and controls. Mann Whitney U test was used for comparison of asthma patients and controls. Correlations between parameters were studied using Pearson's test. Values are expressed as mean ± standard error of the mean (SEM). A significance level (p value) of five percent was used unless stated otherwise.

Results

Of the 14 patients, 11 were atopic and three nonatopic. Mean ages of the patients and controls were 9.0 ± 1.0 and 10.1 ± 1.0 years, respectively ($p > 0.05$). Pulmonary functions were measured in 11 patients. The mean of FEV1 as % predicted was 53.3 ± 4.3 at asthma attack and 95.0 ± 4.1 at remission ($p < 0.001$). The mean of PaO_2 was also significantly lower at asthma attack (69.8 ± 1.5 mmHg) compared to remission (103.0 ± 4.3 mmHg) ($p < 0.001$).

Lipid Peroxides: Patients had significantly higher serum TBARS at asthma attack (415 ± 0.18 μM) compared to remission (2.90 ± 0.15 μM) ($p = 0.001$) (Fig. 1). The mean of controls was 2.2 ± 0.2. The difference between levels during the remission period and in controls was also significant ($p = 0.018$) (Fig. 1). There was a great deal of overlap in the values of these two groups.

Antioxidant Vitamins: Serum levels of ascorbic acid and alpha tocopherol did not disclose any statistical significance between asthma attack and remission (Fig. 2a, 2b). Beta carotene, however, was higher in the remission period compared to the asthma attack ($p = 0.03$) (Fig. 2c). Comparison of antioxidant vitamin levels between the remission period of asthmatic patients and in controls revealed highly

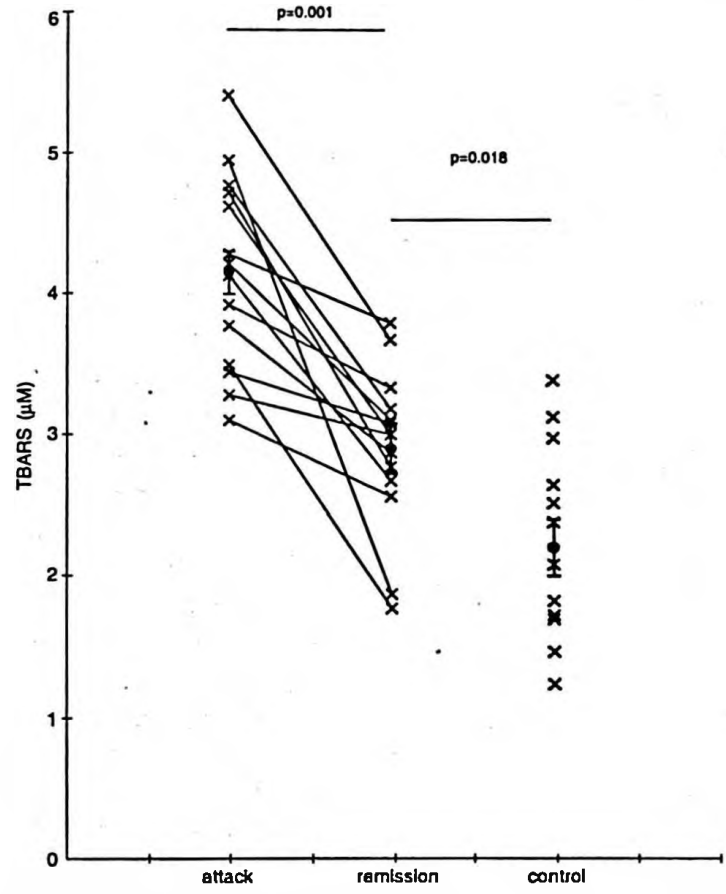

Fig. 1. Levels of thiobarbituric acid reactive substances (μM TBARS) in sera of asthma patients and controls. (-) and error bars denote mean ± SEM.

significant differences for all three vitamins studied (Fig. 2). Interestingly, in asthmatic patients the levels of ascorbic acid, β carotene, and α tocopherol correlated significantly with each other, with r values ranging between 0.89 and 0.95 (Fig. 3). No correlation was found between any of the vitamins and TBARS, PaO_2 or FEV1 ($p > 0.05$ for each).

Discussion

The results of this study confirm previous observations that there is an oxidative stress in asthma and extend further the concept that this oxidative stress is associated with significantly decreased levels of all three antioxidant vitamins, (ascorbic acid, β carotene, and α tocopherol). Although both FEV1 and PaO_2 returned to normal levels, the level of TBARS was still statistically higher compared to controls even after the acute asthma attack subsided. However, it should be noted that despite this statistical significance there is a great deal of overlap between the TBARS values obtained in the remission period with those obtained in the control group.

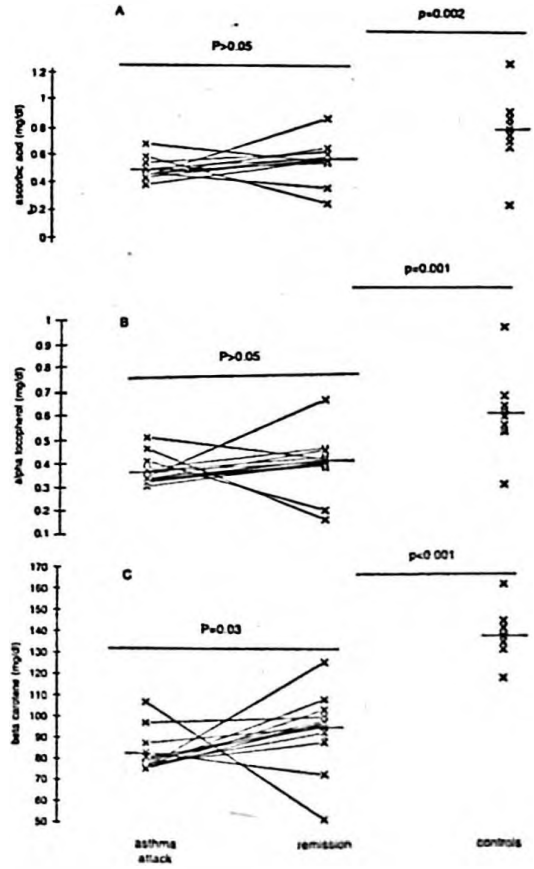

Fig. 2. Antioxidant vitamin levels in sera of asthma patients and controls: – shows the mean for each set of values.

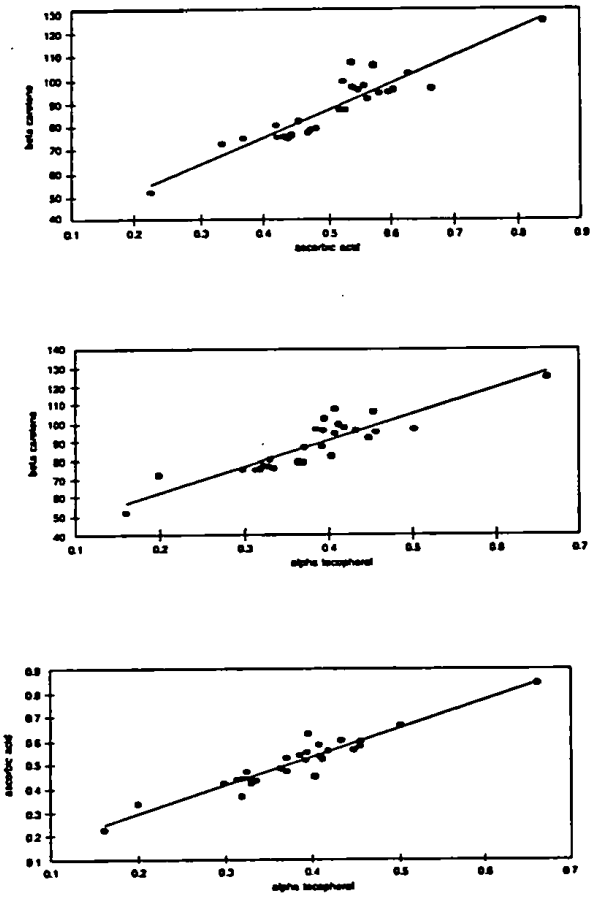

Fig. 3. Correlations among the three antioxidant vitamins studied.

The levels of all three antioxidant vitamins were lower in asthmatic patients compared to controls. One possible explanation for the low levels of vitamins is that the chronic lung disease may have caused decreased dietary intake of vitamins. This seems unlikely, as none of the patients had any evidence of nutritional deficiency. A more likely explanation would be that these vitamins are consumed at an increased rate as a defense mechanism of the organism against the ongoing oxidative burden. This explanation is also not totally satisfactory, as no correlation was observed between any of the vitamins and TBARS, FEV1 or PaO_2. Furthermore, there was no recovery in the low vitamin levels after the hypoxia abated. There are probably some other factors involved that cause the decreases in antioxidant vitamins. The results of this study do not elucidate a definitive cause-effect relationship between the antioxidant vitamins and oxidative stress.

The number of studies that have investigated the relationship between lung functions and antioxidants is quite limited. Vitamin C has attracted relatively more attention in this regard. In accord with our study, Aderele et al.[27] showed that asthmatic children had significantly lower vitamin C levels than controls. Data about the effect of vitamin C on lung function are contradictory, with some showing improvement[28-31] and some showing no effect[32-34]. Two recent studies investigated the role of dietary antioxidants using a questionnaire-based methodology[19,35]. Dow et al.[19] reported that dietary in take of vitamin E may influence lung function inthe elderly, but failed to find such an effect for vitamin C. Soutar et al.[35], in contrast, found that low intake of vitamin C, but not of β carotene or vitamin E, is associated with increased bronchial reactivity.

The imbalance between antioxidant and prooxidant forces has recently been shown in an animal model of asthma[18]. Investigators have shown that vitamin E and ascorbic acid are decreased by 8-and 4-folds in the bronchoalveolar lavage fluid of sensitized animals, and that these decreases are accompanied by a 2.4-fold higher concentration of lipid peroxidation products. The findings of this study support the idea that there is in fact a strong relationship between antioxidant vitamins and oxidant stress.

The observation of very strong correlations among all three antioxidant vitamins in our study strongly suggests that their involvement is most likely mediated via the same mechanism. Our study raises the interesting question of whether or not vitamin supplementation could be of any value in prevention or treatment of childhood asthma, especially in the early stages of disease. Animal studies are now under way to provide some clues to this question.

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