

Comparison of plasma and leukocyte vitamin C status between asthmatic and healthy subjects

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مقارنة بين المصابين بالربو والأصحاء من حيث وضع الفيتامين سي في البلازما وكريات الدم البيضاء
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الخلاصة: وفي هذه الدراسة للحالات والشواهد، أُجريت مقارنة بين وضع الفيتامين سي في 50 من البالغين المصابين بالربو المزمن المسيطر عليه، ووضع في 50 من الأصحاء الشواهد الذين تم انتقاؤهم عشوائياً. وتم تقييم دَخْل الفيتامين سي عن طريق تذكر القوت الذي تناوله كل شخص على مدى ثلاثة أيام، كما تم قياس تركيز الفيتامين سي في البلازما وفي كريات الدم البيضاء بالمقياس اللوني. ولوحظ ترابط إيجابي يُعْتَدُّ به إحصائياً، بين تركيز الفيتامين في البلازما وبين دَخْل هذا الفيتامين في الطعام الذي يتناوله الشخص. وكانت مستويات الفيتامين سي في البلازما وكريات الدم البيضاء منخفضة بشكل يُعْتَدُّ به إحصائياً في المجموعة المصابة بالربو. ولوحظ أيضاً نقص الفيتامين سي في البلازما (أقل من 0.4 مغ/دل) في عدد أكبر من المرضى بالمقارنة مع الشواهد (38% مقابل صفر %). كما لوحظ نقص الفيتامين سي في كريات الدم البيضاء (أقل من 20 ميكروغرام/10⁸ كرية دم بيضاء) في 92% من المصابين بالربو مقابل 8% من الشواهد. وتدلُّ الدراسة على وجود ترابط يُعْتَدُّ به إحصائياً بين مدة حدوث الربو وبين مستوى الفيتامين سي في البلازما.

ABSTRACT In a case-control study the vitamin C status of 50 adults with chronic controlled asthma was compared with that of 50 randomly selected healthy controls. Vitamin C intake was assessed by 3-day dietary recall, and plasma and leukocyte vitamin C concentrations were measured colorimetrically. A positive significant correlation was found between plasma vitamin C and dietary vitamin C intake. Plasma and leukocyte vitamin C levels were significantly lower in the asthma group. Plasma vitamin C was deficient (< 0.4 mg/dL) in significantly more patients than controls (38.0% versus 0%) and leukocyte vitamin C (< 20 µg/10⁸ leukocytes) was deficient in 92.0% of asthmatics versus 8.0% of controls. A significant association was observed between duration of asthma and plasma vitamin C level.

Comparaison du statut en vitamine C plasmatique et leucocytaire entre des patients asthmatiques et des sujets sains

RÉSUMÉ Dans une étude cas-témoins, le statut en vitamine C de 50 adultes asthmatiques chroniques ayant un asthme contrôlé a été comparé à celui de 50 témoins sains choisis au hasard. L'apport en vitamine C a été évalué par rappel des 24 heures sur 3 jours et les concentrations plasmatiques et leucocytaires en vitamine C ont été mesurées par colorimétrie. Une corrélation significative positive a été trouvée entre la vitamine C plasmatique et l'apport alimentaire en vitamine C. Le taux de vitamine C plasmatique et leucocytaire était significativement moins élevé dans le groupe des patients asthmatiques. Il y avait une déficience en vitamine C plasmatique (< 0,4 mg/dL) chez un nombre significativement plus important de patients que de témoins (38,0 % vs 0 %) et en vitamine C leucocytaire (< 20 µg/10⁸ leucocytes) chez 92,0 % d'asthmatiques contre 8,0 % de témoins. Une association significative a été observée entre la durée de l'asthme et le taux de vitamine C plasmatique.

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Introduction

Asthma is a chronic relapsing inflammatory disorder of the airways and a major health problem worldwide [1]. The prevalence and morbidity rate of asthma have increased in the past decade, despite improved knowledge about its pathophysiology and treatment. As inflammatory cells generate and release reactive oxygen species, asthmatic airways are liable to oxidative stress [2]. Moreover, inflammatory cells from asthmatic patients generate more reactive oxygen species than those from controls. The extent of oxidative stress will depend, in part, on the antioxidant defences available within the respiratory tract lining fluid [3].

It has been suggested that antioxidants might have an etiologic role in asthma and, if so, this could lead to the development of new therapeutic strategies. The data are strongest for vitamin C, which is one of the key antioxidant vitamins [4]. It is abundant in the extracellular fluid lining the lung, and adequate vitamin C intake has been associated with the protective effects of airways responsiveness and lung function [1,5–7]. Vitamin C reduces the number and severity of attacks in patients with asthma and reduces the severity of the bronchial responses to exercise [8]. In the search for a possible relationship between vitamin C and asthmatic symptoms, both plasma and leukocyte levels of vitamin C have been studied [9–13]. There are hints that asthmatic subjects have low plasma and leukocyte concentrations of vitamin C but the relationship between vitamin C levels and duration of asthma has not been demonstrated [10–12]. On the other hand, the epidemiological evidence about the role of dietary vitamin C in asthma is controversial [10,11].

No studies of the role of vitamin C in asthma have yet been conducted in the Is-

lamic Republic of Iran. To further investigate the subject, we made a case-control study in Tehran with the hypothesis that plasma and leukocyte vitamin C level are lowered in asthmatic patients and that the duration of asthma affects plasma and leukocyte vitamin C levels.

Methods

A case-control study was conducted to compare the vitamin C status of asthmatics and healthy people at Massih Daneshvari Hospital in Tehran, the Islamic Republic of Iran, between October 2002 and January 2003.

Subjects

Among the asthmatic patients attending the hospital, 50 non-smoking asthmatic patients (24 men and 26 women) were randomly selected using a random number table. Their mean [standard error (SE)] duration of asthma was 8.0 (1.3) years. The diagnosis of asthma was established from a documented clinical history of recurrent cough and/or wheezing and previous demonstration of improvement of symptoms in response to asthma medication. Subjects were selected after each patient had been assessed clinically. The asthma was considered to be under control if no changes were deemed necessary to their current medication after consultation. Patients who had taken oral steroids in the 4 weeks before the study were excluded. All patients were intermittently taking inhaled short-acting beta-adrenergic agents as the sole treatment. These patients were under observation and followed up through periodic visits in the hospital for 2 months.

Among all of the hospital employees, 50 healthy non-smoking persons (26 men and 24 women) were selected for a control group. None of the controls was taking vi-

tamin supplements and none had clinical evidence of an acute respiratory infection.

All of the subjects (patients and controls) were matched for age, weight, height and other variables 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.

Questionnaires

A single 3-day dietary recall questionnaire which has been validated previously against weighed food intakes and in epidemiological studies of heart disease was used to estimate dietary intake [14,15]. This questionnaire was administered by trained personnel possessing at least a bachelor's degree in food and nutrition. The 3-day dietary recall was conducted using a standardized protocol and 51 3-dimensional models to estimate portion size. Interviewers coded the dietary recall questionnaires using nutrient information obtained from the US Department of Agriculture (USDA).

The recommended dietary allowance (RDA) of vitamin C is 60 mg daily and < 45 mg (i.e. less than 75% of this level) is recognized as deficient intake and ≥ 45 mg is recognized as normal intake according to the National Health and Nutrition Examination Survey II (NHANES II) [16].

A sociodemographic and clinical questionnaire that has been extensively validated in our department and elsewhere [14] was completed for each subject; this collected information about sex, literacy, employment, income, suspected risk factors for asthma, other respiratory and allergic disease and drugs used.

Laboratory analyses

Non-fasting blood samples (10 cm³) were drawn from the antecubital space of the

forearm (2 cm³ into small tubes containing EDTA and 8 cm³ into large tubes containing a separator solution). The plasma was separated by centrifugation of the small tubes at 1000 g for 10 min at 4 °C and plasma was pipetted into separate, capped vials for vitamin C analyses. To isolate the leukocytes, after precipitation of the red blood cells, the supernatant was centrifuged at 800–1000g for 5 min in large tubes and the precipitate was washed with distilled water and saline solution. Plasma and leukocyte samples were deproteinized with a trichloroacetic acid solution for the vitamin C assays.

Vitamin C in the protein-free supernatant was determined by a colorimetric method using 2,4-dinitrophenylhydrazine [17]. The cut-off values for deficient plasma and leukocyte vitamin C levels were < 0.4 mg/dL and < 20 μ g/10⁸ leukocytes respectively, according to NHANES II [16].

Statistical analyses

Dietary records were analysed using *Food processor II* software [18,19] and statistical analysis was carried out using *SPSS for Windows*, version 9. The *t*-test was used to compare age, weight, height, daily dietary vitamin C and plasma and leukocyte vitamin C between groups. The chi-squared and Fisher tests were used to compare categorical data (sex, literacy, employment). Pearson correlation was used for determining the correlation between quantitative variables. Simple regression analyses were used to determine whether age, sex or duration of asthma were possible predictor variables with respect to plasma vitamin C levels.

Results

The baseline characteristics of the 2 groups confirmed that they were well matched

(Table 1). The mean (SE) age of patients with asthma was 44.6 (2.2) years and of controls was 44.3 (2.1) years. There were no significant differences in weight, height or age. The frequency distribution of the participants in terms of sex, employment and literacy were similar and showed no significant differences between the 2 groups. The health status indicated that the subjects were different only with respect to asthma and had no other chronic diseases.

Although there was no significant difference in daily dietary vitamin C intake between the 2 groups, there was a significant difference in mean (SE) plasma vitamin C concentration: 0.7 (0.006) mg/dL for asthmatics versus 1.15 (0.006) mg/dL for controls ($P < 0.001$) (Table 1). There were also significant differences in the mean (SE) leukocyte vitamin C levels: 9.6 (1.1) $\mu\text{g}/10^8$ leukocytes for the asthma group and 31.2 (7.5) for the controls ($P < 0.001$) (Table 1).

The distribution of participants by vitamin C status showed that plasma vitamin C was deficient in 38.0% of patients (< 0.4 mg/dL) compared with 0% of controls (P

< 0.001) (Table 2). Leukocyte vitamin C was deficient ($< 20 \mu\text{g}/10^8$ leukocytes) in 92.0% of patients, but only 8.0% of controls ($P < 0.001$).

The mean daily dietary intake of vitamin C was deficient ($< 75\%$ of RDA) in 50.0% of the asthma group and 32.0% of controls, although the difference between groups was not significant (Table 2). Plasma vitamin C was deficient in 47.4% of women and 52.6% of men, and leukocyte vitamin C was deficient in 52.0% of women and 48.0% of men. There was no significant association of sex, health status, literacy, employment or severity of asthma (mild or severe) with plasma or leukocyte vitamin C status.

Table 3 compares the percentage of participants with deficient/normal vitamin C levels and deficient/normal vitamin C intake. There was a significant association between plasma vitamin C status and dietary vitamin C intake in the 2 groups combined ($P < 0.03$) (Table 3), but leukocyte vitamin C status had no significant association with dietary vitamin C intake.

Table 1 Baseline characteristics of participants

| Variable | Asthma group (n = 50) Mean (SE) | Control group (n = 50) Mean (SE) |
|--|---------------------------------------|--|
| Age (years) | 44.6 (2.2) | 44.3 (2.1) |
| Weight (kg) | 67.8 (1.4) | 68.4 (1.3) |
| Height (cm) | 165.7 (1.0) | 166.9 (1.0) |
| Vitamin C status | | |
| Plasma vitamin C level (mg/dL) | 0.7 (0.006)*** | 1.15 (0.006) |
| Leukocyte vitamin C level ($\mu\text{g}/10^8$ leukocytes) | 9.6 (1.1)*** | 31.2 (7.5) |
| Vitamin C daily intake (mg) | 71.1 (9.8) | 82.8 (7.5) |

*** $P < 0.001$, independent t-test.
SE = standard error.

Table 2 Frequency distribution of participants in terms of plasma and leukocyte vitamin C status and dietary vitamin C intake

| Vitamin C status | Asthma group (n = 50) | | Control group (n = 50) | |
|--------------------------------------|--------------------------|------|---------------------------|-------|
| | No. | % | No. | % |
| <i>Plasma vitamin C status***</i> | | | | |
| Deficient | 19 | 38.0 | 0 | 0 |
| Normal | 31 | 62.0 | 50 | 100.0 |
| <i>Leukocyte vitamin C status***</i> | | | | |
| Deficient | 46 | 92.0 | 4 | 8.0 |
| Normal | 4 | 8.0 | 46 | 92.0 |
| <i>Vitamin C intake</i> | | | | |
| Deficient | 25 | 50.0 | 16 | 32.0 |
| Normal | 25 | 50.0 | 34 | 68.0 |

***P < 0.001, Fisher test.

A significant positive correlation was observed between plasma vitamin C level and dietary vitamin C intake in the asthma group ($r = 0.49$, $P < 0.001$) and in the control group ($r = 0.55$, $P < 0.001$) (Table 4). A significant positive correlation was also found between plasma vitamin C level and leukocyte vitamin C level in the control group ($r = 0.47$, $P < 0.01$).

Regression analysis showed that the age of asthmatics and control subjects had no

association with plasma and leukocyte vitamin C but duration of asthma had a significant association with plasma vitamin C level ($P < 0.03$). Therefore each unit increase in duration of asthma affliction (i.e. each year of increase) was associated with 0.024 mg/dL decrease in mean plasma vitamin C in the asthma group but these changes was not significant for leukocyte vitamin C levels.

Table 3 Frequency distribution of plasma and leukocyte vitamin C status in relation to dietary vitamin C intake

| Vitamin C intake | Plasma vitamin C status | | Leukocyte vitamin C status | | Plasma vitamin C status | | Leukocyte vitamin C status | |
|------------------|-------------------------|---------|----------------------------|--------|-------------------------|--------|----------------------------|--------|
| | Deficient* | Normal* | Deficient | Normal | Deficient | Normal | Deficient | Normal |
| | No. | % | No. | % | No. | % | No. | % |
| Deficient | 12 | 63.2 | 29 | 35.8 | 23 | 46.0 | 18 | 36.0 |
| Normal | 7 | 36.8 | 52 | 64.2 | 27 | 54.0 | 32 | 64.0 |
| Total | 19 | 100.0 | 81 | 100.0 | 50 | 100.0 | 50 | 100.0 |

*P < 0.05, Fisher test.

Table 4 Correlation between plasma and leukocyte vitamin C levels and dietary vitamin C intake in the 2 groups of participants

| Vitamin C level | Vitamin C intake | |
|---------------------------|------------------|------------------|
| | Asthma group | Control group |
| Plasma vitamin C level | $r = 0.49^{***}$ | $r = 0.55^{***}$ |
| Leukocyte vitamin C level | $r = 0.2$ | $r = -0.1$ |

*** $P < 0.001$, Pearson's correlation, plasma vitamin C versus dietary intake.

Discussion

Vitamin C, as an important antioxidant, has been hypothesized to have a role in preventing the increased production of oxidants in asthma and the resulting oxidative stress in the lungs [2,11]. Our study showed that mean plasma and leukocyte vitamin C concentrations in a group of asthma patients were significantly lower than a control group. This was consistent with the findings of other studies [20–24]. In our asthma group, 38% and 92% of participants had deficient levels of vitamin C in plasma and leukocytes respectively, compared with 0% and 8% of controls.

We also showed a significant positive correlation between plasma vitamin C status and dietary vitamin C intake in the 2 groups. 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 intake of vitamin C 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 [12,16,22], so leukocyte vitamin C would be a more sensitive indicator of duration of asthma. This is consistent with our findings showing a greater difference in leukocyte than plasma vitamin C between groups. 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 [3].

Vallance et al. reported that subjects who have undergone infarction, infection and surgery had leukocytosis complications that result in a decrease in leukocyte vitamin C [26]. In the present study, none of the patients had any chronic disease (except asthma) or history of surgery, so there was no leukocytosis due to other diseases. The results of this study were consistent with Olusi [21] and Aderole [22]. Olusi et al. found significantly higher concentrations of vitamin C in plasma and leukocytes in controls than in either treated or untreated patients with asthma [21]. Similarly, in a study comparing plasma vitamin C levels in children with asthma with levels in healthy controls, Aderole et al. found significantly lower levels in children with asthma. However, no relationship was demonstrated between vitamin C levels and severity or duration of asthma. Unfortunately, Aderole's study did not quantify vitamin C intake in the diet or in supplements taken by approximately 50% of the children, making the results difficult to interpret [22].

Schwartz and Weiss assessed the relationship between dietary vitamin C intake and pulmonary function in 2526 randomly selected adults, of whom approximately 3% were asthmatic [23]. Lower dietary vitamin C intakes were shown to be directly related to lower plasma vitamin C levels and

lower forced expiratory volume at 1 second (FEV₁). Although the accuracy of dietary recall may be low in their survey, the population surveyed was large and the margin of error was small.

Other studies showed that lower intake of vitamin C was associated with the presence of respiratory symptoms and pulmonary diseases such as asthma [1,11,24–26]. In the present study, the 3-day dietary recall showed 50% of asthma patients reporting deficient vitamin C intake (below the RDA) and 50% with vitamin C intake above the RDA. But this study was done in autumn when the availability of citrus fruits is high and repeating the study in other seasons might show different results.

Powell compared antioxidant status in 35 asthmatic patients with 35 healthy subjects and reported that there was no significant difference in plasma vitamin C between the 2 groups, which was inconsistent with the results of the present study. However, in Powell's study, only plasma vitamin C and not leukocyte vitamin C was measured and all the subjects were infants [27]. In our study, the asthmatic patients were taking inhaled short-acting beta-adrenergic agents intermittently as the sole treatment, but neither these drugs nor oral corticosteroids have been observed to affect vitamin C status in asthmatic patients [3,27].

Vural and Uzun reported that red blood cell vitamin C levels in asthmatic patients were significantly lower than a control group, but smoking status, employment, other diseases and leukocyte vitamin C

were not considered [28]. Trenga et al. in a double-blind crossover study reported that vitamin C plus vitamin E can decrease the ozone-induced bronchial hyper-responsiveness in adults with asthma [29].

Some potential limitations of our study are the small sample size, measuring vitamin C by the colorimetric method, which is less sensitive than high-pressure liquid chromatography, and the timing of the study in autumn when the intake of citrus fruits as a rich source of vitamin C is good.

In conclusion, this study showed that both plasma and leukocyte vitamin C levels are significantly lower in asthmatic patients compared with healthy subjects. Leukocyte vitamin C reduction was greater in asthmatic patients. Plasma vitamin C levels were more influenced by diet. These findings, which may have important scientific and public health implications, justify a more detailed and larger prospective study of the effects of disease activity and treatment on vitamin C status. Ongoing and proposed interventional studies are needed to define the role of vitamin C supplementation in asthma and to define indications to support its ongoing use.

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International statistical classification of diseases and health related problems, 10th revision, (ICD-10), 2nd edition

This new edition of WHO's *International statistical classification of diseases and related health problems*, 10th revision (ICD-10) has been fully updated. Originally published in the early 1990s, ICD-10 now incorporates all updates and other changes to this core health classification since 1996. The new edition has hundreds of updates, and includes new diseases such as SARS, to ensure that the classification is entirely suited to today's needs. Improvements have been made to the rules and guidelines for mortality coding, making them easier to implement and which will result in the improved comparability of international mortality statistics. In addition, there are many additions and changes to the alphabetical index resulting in some 60 additional pages, making it far more comprehensive than that of the first edition. The three-volume second edition follows the same structure as the first edition, with the addition of a chapter XXII (Codes for special purposes) made necessary by the emergence of SARS and the need for an interim solution for the coding of this major health problem. The ICD-10 2nd edition is available electronically on CD and as a downloadable version. Further information on this publication can be obtained from WHO Press: <http://www.who.int/bookorders/anglais/home1.jsp?sesslan=1>