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Ascorbic Acid Concentration in Plasma and White Blood Cells of Patients with Bronchial Asthma

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ABSTRACT

Background: Asthma is an inflammatory disease of the airways. Oxidants are of the important factors damaging the airways. Moreover, an inappropriate correlation exists between oxidants and antioxidants in asthma. Vitamin C is one of the major protective antioxidants of the airways. Thus, we evaluated the concentration of Vit C in plasma and white blood cells of asthmatic patients.

Materials and Methods: A case-control study was performed on 50 asthmatic patients referred to Masih Daneshvari Hospital. Data were collected through the general information and 24h dietary recall questionnaires and then the effect of independent variables on plasma and WBC ascorbic acid concentrations was evaluated by statistical analyses using biochemical tests.

Results: Data showed that plasma and WBC ascorbic acid deficiency exists in 38% and 92% of asthmatic patients, respectively. There was a significant difference in plasma and WBC Vit C concentrations between case and control groups ($p < 0.0001$). There was a positive significant correlation between the level of plasma ascorbic acid and dietary Vit C intake ($p = 0.0001$).

Conclusion: The present study showed a relationship between asthma and ascorbic acid levels in plasma and in WBCs. More precise studies are recommended for better determination of asthma and vitamin C correlation. (*Tanaffos* 2006; 5(4): 29-35)

Key words: Vitamin C, Ascorbic acid, Asthma, Plasma, White Blood Cell

INTRODUCTION

There is a direct correlation between reduced pulmonary function and mortality in the general population (1-3). Numerous studies have confirmed

the positive effects of antioxidant vitamins including vitamin C (4-9), Vit E and β -carotene on pulmonary function. However, there is insufficient evidence in this regard (10).

Asthma is primarily an inflammatory disease, and the bronchial airways have been shown to be particularly susceptible to oxidant-induced tissue damage (11).

Vit C is the most essential antioxidant within the

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lung lining fluid of the airways and is a protective factor against external oxidants such as cigarette smoke and environmental pollutants. On the other hand, reactive oxygen derivatives released from eosinophils, macrophages and neutrophils during an asthma attack, have an important role in the development of disease. They directly induce contraction of smooth muscles of the airways and stimulate histamine release. There are oxidant-anti oxidant imbalances as well as oxidative stress conditions in asthmatic patients (12).

In developed countries where dietary regimen has gradually lost proper amounts of fresh vegetables and fruits, pulmonary diseases induced by harmful inhaled materials are more common (13). Based on Schwartz et al. study, high levels of plasma Vit C were accompanied by lower incidences of bronchial inflammation and wheezing (14). Fogarty et al. reported that epidemiologic studies considered Vit C as an effective factor in reducing symptoms of asthma (15).

A study conducted by Olusi et al. in 1979 showed that the plasma level of Vit C in patients with asthma was different from that of healthy controls (16). There are controversial studies about the effect of Vit C on asthma (17).

Due to the lack of sufficient evidence required to confirm or rule out the effect of Vit C on respiratory symptoms of the patients with asthma, we decided to evaluate its effect on symptoms of these patients.

MATERIALS AND METHODS

A case-control study was conducted in 2001 to evaluate the level of ascorbic acid in plasma and white blood cells of asthmatic patients referred to Masih Daneshvari Hospital (Tehran). In this study, asthmatic patients who did not have history of smoking, myocardial infarction or infection were evaluated.

Healthy non-smokers with no history of smoking were also selected as the control group. The subjects

received general questionnaire and filled the 24h dietary recall (18) and underwent biochemical tests including plasma and WBC ascorbic acid measurements. In this survey, asthmatics whose disease had been confirmed by a pulmonologist and had been present for 6 months, were selected randomly. Afterwards, general questionnaires including general information (age, gender, etc) were filled out via interview and then blood samples were taken from subjects. Since the concentration of Vit C in both plasma and white blood cells had to be measured, 10 cc of non-fasting venous blood was taken from each patient. A 24-hour dietary recall questionnaire was also filled. Ascorbic acid intake was measured as mg by FPII (Food Processor II, ESHA research, Salem, Oregon, 1987).

Blood samples were separated into two groups of plasma and WBC and transferred into prepared tubes for further examination. Lowry method was used to measure the Vit C level in plasma and WBC, separately.

Finally, colorimetry of the prepared samples was performed within 24 to 48 hours after freezing.

Measurement of the level of ascorbic acid in white blood cells:

Principles:

Whole blood was mixed with a solution containing saline, dextran and ethylene diamine tetraacetic acid (EDTA) in which red blood cells (RBCs) immediately precipitated by rouleaux formation. The remaining WBCs were precipitated by separating and centrifuging the upper layer. Subsequently, the amount of their ascorbic acid, extracted by trichloroacetic acid, was measured by 2, 4 dinitro-phenyl-hydrazine method.

Indicators:

- 1- Precipitating RBC solution (the following solutions were mixed as 200/50/2 ratios):
 - Sodium chloride 8.5 g/L
 - Dextran 60 g/L
 - Sodium EDTA 100 g/L

- 2- Trichloroacetic acid 50 g/L
- 3- Staining solution (the following solutions were mixed as 20/1/1 ratios):
 - 2,4 dinitrophenyl hydrazine (in sulfuric acid) 10 mol/L
 - Thiourea 50 g/L in distilled water
 - Cupric (Cu) sulfate 6 g/L in distilled water
- 4- Sulfuric acid solution (650 cc sulfuric acid was gradually added into 350cc distilled water).
- 5- Standard ascorbic solution 10 mg/L (in solution No.2)

Method:

Six to 10 cc of venous blood sample was added to 12.5cc of precipitating solution and stored for 30 minutes. Subsequently, 10cc of the upper layer was transferred into another tube and centrifuged at 3000 rpm for 15 min. The upper layer was thrown away. 1.3cc of trichloroacetic acid was added into precipitated WBCs and the remaining solution was centrifuged. Then, 1cc of superficial solution was transferred into the test tube. One milliliter of standard solution and 1 cc of trichloroacetic acid solution were added to std and blank tubes, respectively. Staining solution 0.3 cc was added to all tubes and incubated at 37°C for 4 hours. Finally, 2 cc of sulfuric acid was added into all tubes, mixed and read with a 520 nm spectrophotometer (19).

RESULTS

Data showed that there were no significant differences in sex distribution ($p=0.84$) and health status (with or without asthma) between the case and control groups ($p= 1$, using Fischer's test). There was no statistically significant difference in drug consumption between the two groups.

Table 1 is indicative of plasma ascorbic acid status. It showed that 38% of patients had a mean plasma Vit C less than 0.4 mg/dL (below the standard level) and 62% were within the normal

range. In the control group, all individuals had normal levels of plasma Vit C (there was a significant difference between the case and control groups).

Table 1. Frequency of study subjects according to the level of plasma ascorbic acid.

Plasma ascorbic acid		Study group		Total
		Case	Control	
Deficiency	No.	19	0	19
	(%)	38	0	19
Normal	No.	31	50	81
	(%)	62	100	81
Total	No.	50	50	100
	(%)	100	100	100

Deficiency: Plasma Vit C<0.4 (mg/L)

Normal: Plasma Vit C>0.4 (mg/dL)

Table 2 shows the frequency distribution of subjects based on WBC ascorbic acid levels. It indicated that 92% of patients had WBC ascorbic acid levels less than 20 $\mu\text{g}/10\text{WBC}$, whereas 8% of the controls showed this deficiency. Eight percent of patients and 92% of the controls had a normal level of WBC ascorbic acid. There was a significant difference between the patient and control groups ($p<0.0001$), using Fischer's test.

Table 2. Frequency of study subjects according to the level of WBC ascorbic acid.

WBC ascorbic acid		Study group		Total
		Case	Control	
Deficiency	No.	46	4	50
	(%)	92	8	50
Normal	No.	4	46	50
	(%)	8	92	50
Total	No.	50	50	100
	(%)	100	100	100

Frequency distribution of subjects based on ascorbic acid status is shown in table 3. Fifty percent of patients had dietary intake of Vit C less than

45mg/day [less than 75% of recommended dietary allowance (RDA)], and the remaining 50% received 45 mg/day or more.

Table 3. Frequency of study subjects regarding dietary ascorbic acid intake.

Dietary ascorbic acid		Study group	
		Case	Control
Deficiency (<45mg/day)	No.	25	16
	(%)	50	32
Normal (\geq 45mg/day)	No.	25	34
	(%)	50	68
Total	No.	50	50
	(%)	100	100

In contrast, 32% of the controls had dietary Vit C intake less than 75% of the RDA recommended dose. However, the remaining 68% had normal intake of Vit C which was not statistically significant.

Table 4 shows the comparison of the means of quantitative variables in case and control groups. There was a significant difference in the means of plasma and WBC ascorbic acid concentrations in the case and control groups ($p < 0.0001$). The mean (\pm SD) duration of asthma was $8(\pm 1.3)$ yrs. Totally, 47.4% of women and 52.6% of men had plasma ascorbic acid deficiency while 52% of women and 48% of men had WBC ascorbic acid deficiency. There was no significant difference between the sex and level of ascorbic acid ($p = 0.84$, $p = 1$).

Table 4. Comparison of the quantitative variables in patient and control groups.

Variable	Patient (n=50)		Control (n=50)		P value
	Mean	SE	Mean	SE	
Weight (kg)	67.8	1.40	68.4	1.3	0.78
Height (cm)	165.7	1.00	166.9	1	0.42
Age (year)	44.6	2.20	44.3	2.1	0.91
Plasma ascorbic acid level (mg/dL)	0.7	0.006	1.15	0.006	0.0001
WBC ascorbic acid level (mg/10WBC)	9.6	1.1	31.2	7.5	0.0001
Dietary Vit C intake (mg)	71.1	9.80	82.8	7.5	0.34
Duration of asthma (year)	8	1.30	-	-	-

73.7% of study subjects were healthy in spite of having plasma Vit C deficiency. There was no significant correlation between the level of ascorbic acid (in plasma and WBC) and health status. ($p = 1$, $p = 0.068$).

Data showed that 89.5% and 74% of study subjects who had used drugs had plasma and WBC Vit C deficiencies, respectively. There was a significant correlation between the level of ascorbic acid (in plasma and WBC) and drug consumption ($p < 0.0001$).

Table 5 shows the level of plasma and WBC ascorbic acid based on dietary ascorbic acid intake. As mentioned before level of plasma ascorbic acid was related to dietary Vit C intake ($p = 0.03$). No significant correlation was found between WBC ascorbic acid and dietary Vit C intake ($p = 0.41$).

Table 5. Level of plasma ascorbic acid based on dietary ascorbic acid intake.

Health status		Plasma ascorbic acid		WBC ascorbic acid		Total (deficiency+normal)
		Deficiency	Normal	Deficiency	Normal	
Healthy	No.	14	73	43	44	87
	(%)	73.7	90.1	86	88	87
Patient	No.	5	8	7	6	13
	(%)	26.3	9.9	14	12	13
Total	No.	19	81	50	50	100
	(%)	100	100	100	100	100

Plasma ascorbic acid deficiency was severe in 57.9% and mild in 42.1% of subjects. Among individuals with WBC ascorbic acid deficiency, 41.3% and 58% showed severe and mild deficiencies, respectively. There was no significant correlation between the severity of disease and the

level of ascorbic acid ($p=0.07$, $p=0.6$).

Regression analysis showed that the patients age had no correlation with plasma and WBC ascorbic acid concentrations ($p=0.549$, $p=0.667$).

On the other hand, regression coefficient was 0.0248 (SE=0.01) for duration of asthma ($p=0.03$). This means that the longer the duration of disease, the greater the decrease in mean plasma Vit C concentration (to 0.024 mg/dL) in the patient group. This relationship did not exist in WBC ascorbic acid level and was not significant ($p=0.51$).

Regression analysis showed that the age of healthy subjects had no effect on plasma and WBC ascorbic acid levels ($p=0.11$).

DISCUSSION

According to the obtained results, we concluded that asthma was correlated with plasma and WBC ascorbic acid levels. Although ascorbic acid level was not influenced by other independent variables including sex, level of education, occupation or health status in this study, we can not suggest that the disease is influenced by the level of Vit C, and further researches are necessary to find a correlation between asthma and level of ascorbic acid.

As shown in our study, only 38% of subjects had plasma ascorbic acid deficiency and no deficiency was detected in the control group. In the patient group, the majority had normal concentration of plasma Vit C. However, there was a significant difference in plasma and WBC ascorbic acid levels between the patient and control groups.

Severe deficiency of WBC ascorbic acid was detected in 92% of subjects in the patient group. The results showed that WBC ascorbic acid in patients was more influenced by the disease as compared to plasma ascorbic acid. This may be due to the fact that plasma Vit C is more influenced by the dietary intake of vitamin.

Erroneous measurement of plasma Vit C may

complicate the analysis of plasma ascorbic acid. Therefore, measurement of WBC Vit C concentration is indicative of Vit C status in longer periods of time (20-22).

Data showed that the study subjects did not have problems with the dietary intake of Vit C. A study conducted by Vallance (23) in 1979 demonstrated that the level of WBC ascorbic acid may be falsely low in subjects with leukocytosis induced by myocardial infarction, infection and/ or surgery. The present study did not show this fact. Our results were consistent with those of other studies. In 1979, Olusi et al. (16) detected Vit C deficiency in plasma and WBCs of patients.

In 1985, Aderole et al. showed that mean of plasma Vit C in asthmatic patients was lower than in controls (24). Other studies regarding the correlation of dietary Vit C intake with respiratory diseases showed that lower amount of dietary Vit C intake was accompanied by an increasing incidence of respiratory diseases such as asthma (22, 25, 26). Regarding antioxidant status in asthmatics, our findings confirmed the results of the previous studies and showed that Vit C level was lower in asthmatic patients as compared with healthy subjects. In contrast, erythrocyte glutathione and ceruloplasmin levels were higher in the patient group than in controls (27). In a study performed on lung lining fluid of patients with asthma, Vit C and alpha-tocopherol (Vit E) concentrations were lower in patients with mild asthma than in healthy volunteers, even though the blood levels were normal or increased. On the other hand, the amount of oxidised glutathione had been increased in these patients and increased oxidative stress was considered (28).

A case-control study undertaken by Powell et al. in 1994 on 35 patients with asthma for evaluation of antioxidant status showed no significant difference in the level of some of the antioxidants including Vit C between patient and control groups (29). It must be

mentioned that in Powells' study only the plasma Vit C was evaluated and no evaluation was carried out on WBC Vit C. Additionally, the age group consisted of children; thus, researchers suggested that further studies should be done in this regard.

As a conclusion, Vit C status especially WBC ascorbic acid concentration should be examined in patients with asthma because as observed in this study, Vit C reserve is more influenced in this group. Although some drugs used by asthmatic patients (like corticosteroids) have impact on the level of Vit C (13) more extensive studies should be done by evaluating other antioxidants in addition to Vit C in these patients.

We recommend consumption of vegetables, fresh fruits and Vit C supplements in subjects with low level of plasma Vit C, because this vitamin can be effective in lowering the rate of obstructive lung diseases and may even have a preventive effect on etiology of these diseases.

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