

Original Research Article

Plasma ascorbic acid therapy and eosinophilic counts in allergic rhinitis: a pilot study

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ABSTRACT

Background: Role of plasma ascorbic acid in reducing the eosinophilic counts in subjects of allergic rhinitis was studied.

Methods: 60 patients were randomly selected from the Rhinology clinics of Oto- Rhino- laryngology and Head-Neck services of a tertiary health care facility. Shift in the eosinophilic counts in three groups of patients i.e., allergic rhinitis, placebo and control groups was analyzed and compared. The study was undertaken in a period of 1^{1/2} years (June 2009 -January 2010) Blood eosinophilic count as %age of DLC was noted.

Results: The mean eosinophilic count was elevated in allergic patients (8.009±2.91) with respect to that in the controls (4.70±1.26). It reverted to normal with treatment with vitamin C. No change was seen in the placebo group. (5.45±3.56).

Conclusions: Vitamin-c reduces the eosinophilic counts in allergic rhinitis.

Keywords: Allergic rhinitis, Ascorbic acid, Eosinophilic count, Vitamin C

INTRODUCTION

Ascorbic acid helps in blocking the allergic symptoms by reducing the disulphide bonds between the chains of antibody molecules making their binding with antigen impossible. Antibodies seek to match antigens only in the areas where stray free radicals or a relatively oxidizing redox potential exists. The redox state of normal, healthy tissue does not allow the binding of antibody to antigen. When antioxidant, free radical scavenging systems are overwhelmed, inflammatory or hypersensitivity and autoimmune conditions may result.¹⁻³ Aim of the study was to study the effect of vitamin c/ascorbic acid on eosinophilic count in patients of allergic rhinitis.

METHODS

60 patients were randomly selected from the Rhinology clinics of Oto- Rhino- laryngology and Head-Neck services of a tertiary health care facility. Shift in the eosinophilic counts in three groups of patients i.e., allergic rhinitis, placebo and control groups was analyzed and compared. The study was undertaken in a period of 1^{1/2} years (June 2009 -January 2010).

Group 1 was the control group of 20 normal individuals. It was subjected to plasma ascorbic acid estimations.

Group 2 was the experimental group of 40 patients) It was examined during the episode of acute onset of Rhinitis to find any significant difference in the value

with respect to the controls. Simultaneously their complaints, clinical features and examination findings documented on the prescribed proforma to correlate with plasma ascorbic acid levels.

The experimental group of 40 cases was divided into two subcategories:

Group A was Case group of 20 subjects

This group was given 1 gm/day of vitamin – C supplementation for 7 days and plasma levels were estimated after supplementation. Simultaneously their complaints and clinical features were noted and compared with previous findings.

Group B was Placebo group of 20 subjects

This group was given placebo. Placebo consisted of sugar tablet manufactured by Jain Ayurvedic Dawakhana, Sabun Bazaar, Ludhiana (Punjab). After giving placebo twice daily for seven days, the plasma levels were estimated and the clinical manifestation compared. Due care was taken that the subjects did not take any vitamin C supplementation, antihistamines or decongestants prior to plasma level estimation.

Inclusion criteria

Patients with allergic rhinitis were included in the study.

Exclusion criteria

Patients on antihistamines, steroids and vitamin c were excluded from the study.

Methodology

Plasma levels of ascorbic acid was estimated by 2, 6 – dichlorophenol indophenols titration (Bassey, 1944)

Principle

2, 6 – dichlorophenol indophenols is blue compound, which is red in acid solution. On titration with a solution of ascorbic acid the dye is reduced to colorless solution, the ascorbic acid being oxidized to dehydroascorbic acid.

Reagents

Trichloroacetic acid, 10% and Solution of 2, 6 - dichlorophenol indophenols, 1 ml of which is equivalent to 0.02 mg of ascorbic acid. The 5 ml of this solution is diluted to 25 ml. Now 1 ml of this diluted solution is equivalent to 0.04 mg of ascorbic acid (Harris and Ray. 1935), i.e. 1 ml of dye is equivalent to 0.02 mg of ascorbic acid 5 ml of dye is equivalent to 0.01 mg of ascorbic acid. This 5 ml is diluted to 25 ml which now has 0.1 mg of ascorbic acid. So, 25 ml now is equivalent to 0.1 mg of ascorbic acid 1 ml of dilute dye is equivalent to 0.4 mg of ascorbic acid

Method

Under all aseptic conditions 5 ml of ascorbic acid withdrawn from the antecubital fossa. Plasma separated immediately after withdrawing the blood by Centrifugation. The plasma so separated was mixed with equal volume of Trichloroacetic acid (i.e. dilution factor = 2).

The mixture was centrifuged to precipitate proteins and liberate protein bound ascorbic acid. The supernatant separated and preserved. The sediment discarded. 0.2 ml of the diluted dye of 2, 6 - dichlorophenol indophenol solution pipetted out in the test tube. This 0.2 ml of diluted dye is titrated with the supernatant till colorless solution is obtained.

Calculations

0.2 ml of diluted dye is titrated with the supernatant (whose dilution factor was 2 as plasma was mixed with equal volume of tri-chloroacetic acid) i.e. 1 ml of diluted dye is equivalent to 0.04 mg of ascorbic acid, 0.2 ml of diluted dye is equivalent to 0.008 mg of ascorbic acid. Now, 'x' ml plasma titrated with 0.2 ml of diluted dye i.e. x ml plasma used up in titration contained 0.008 mg of ascorbic acid.

$$100 \text{ ml plasma will contain} = \frac{100}{\text{'x' ml titration}} \times 0.008$$

But plasma was diluted with equal volume trichloroacetic acid (to precipitate proteins), therefore, the dilution being 1:2 (Dilution factor = 2). So, 100 ml plasma actually will have:

$$= \frac{100}{\text{'x' ml titration}} \times 0.008 \times \text{Dilution factor}$$

$$= \frac{100}{\text{'x' ml titration}} \times 0.008 \times 2$$

100 ml of plasma will, therefore, have ascorbic acid equivalent to:

$$= \frac{100}{\text{'x' ml titration}}$$

Interpretations

The normal range of plasma ascorbic acid as estimated in our set up by this method (2, 6 - dichlorophenol indophenol titration) is between 0.8 to 2.2 mg per deciliter.

Statistics

All statistical calculations were done using Statistical Package of Social Sciences (SPSS) 17 version statistical program for Microsoft Windows (SPSS Inc. Released 2008. SPSS statistic for windows, version 17.0, Chicago). Ethical approval of the study was taken from the Institutional Ethics Committee.

RESULTS

Table 1: Blood eosinophils counts in various groups (n=60).

Group	Mean eosinophil count (as % of DLC) ±SD
Control group	4.70±1.26
Case group	
Pre vitamin C	8.09±2.91
Post vitamin C	5.45±3.56
Placebo group	
Pre placebo	6.38±3.12
Post placebo	6.30±2.48

Table 2: Absolute eosinophilic counts (n=60).

Group	Mean absolute eosinophil count (109/l)±SD
Control group	0.3105±0.7337
Case group	
Pre vitamin C	0.514±0.5128
Post vitamin C	0.386±0.313
Placebo group	
Pre placebo	0.5511±0.1285
Post placebo	0.5278±0.1149

Table 3: Statistical significance of absolute eosinophil counts in various study groups.

Comparison of total eosinophil counts in groups	t value	Statistical significance of p value
Pre Vitamin C vs. control	5.61	SS at p<0.01
Pre vit. C vs. post Vit. C	3.52	SS at p<0.01
Post Vit.C vs control	0.80	Not significant
Pre Placebo vs. control	6.15	SS at p<0.01
Pre placebo vs. post placebo	0.59	Not significant
Post Placebo vs. control	5.91	SS at p<0.01

The mean eosinophilic count was elevated in allergic patients (8.009±2.91) with respect to that in the controls (4.70±1.26). It reverted to normal with treatment with vitamin C. No change was seen in the placebo group.

(5.45±3.56). The three groups in our study showed the following variation in absolute eosinophil counts. The mean total eosinophilic counts were elevated (0.514±0.5128) in patients of allergic patients as compared with controls. (0.3160) after vitamin C treatment eosinophil counts returned to normal. (0.36) Placebo had no effect. It is clear from the above tables that, there is significant increase in levels of Eosinophils in blood vis-a-vis control. These increased eosinophil levels revert to control level with vitamin C treatment. Placebo has no significant effect in decreasing Eosinophils in blood.

DISCUSSION

Eosinophilic counts

The normal range of blood Eosinophils counts in our study was 4.70±1.26 as percentage of DLC, the normal absolute Eosinophils count was 0.3105±0.7337x109/l.

In the case group blood Eosinophils count before vitamin C treatment was 8.09±2.91% of DLC. The absolute Eosinophils counts respectively were 0.514±0.5128x109/l and 0.386±0.313x109/l.

In the placebo group the blood eosinophil count before vitamin C treatment was 6.38±3.12% of DLC which after vitamin C treatment was 6.30±2.48. The absolute eosinophil counts respectively were 0.5511±0.1285x109/l and 0.5278±0.1149x109/l.

In our study, the patients with allergic rhinitis, 4 patients had eosinophilia of 3% or less (% of DLC), 12 had eosinophilia of 4 to 7% and 19 had eosinophilia of more than 7%. Thacker et al in a study on 170 cases found that blood eosinophilia of 3% or less in 55 cases, 4 to 7% in 30 cases and 8 to 15% in 15 cases.¹ Munjal et al. found blood eosinophilia in 43.3% cases while eosinophilia in nasal smear were present in 50% cases. Out of it 38.4% showed occasional eosinophils in nasal smear.²

They found that ascorbic acid level in normal individual was 1.93 mg% in blood and its excretion in urine was 17.43 mg/day. They reported that in acute allergic rhinitis these levels are much low which denotes unsaturated states of body which is again demonstrated by increased utilization. Heavy doses of ascorbic acid were found to be objectively beneficial in allergic rhinitis.

Schwartz et al confirmed that vitamin C neutralizes free-radicals and suppresses macrophage secretion of superoxide anions.⁴ Nagpal et al carried out a study on 20 normal subjects and 20 patients and found that the mean ascorbic acid level in the control group was 4.15mg/l while it was 3.4 mg/l in the acute phase of tonsillitis.⁵

Bucca et al showed the beneficial effect of oral administration of 2 gm vitamin C on allergic

responsiveness to inhaled histamines in 16 patients with allergic rhinitis.⁶

High concentration of ascorbic acid in leucocytes and its rapid utilization during infection and phagocytosis suggests its role in immune response. This protective role of ascorbic acid in recurrent infection and allergy is attributed to its stimulatory effect on interferon synthesis and lymphocyte function leading to augmented immune response.⁷ Maderazo et al. demonstrated that the antioxidants like vitamin C improve the polymorphonuclear cells locomotion vis-a-vis placebo.⁸

Blood histamine is significantly depressed in high blood ascorbate level and leukocyte chemotaxis is significantly impaired in low blood ascorbate levels.⁹ Jullinson et al showed that the mast cell is an important trigger cell in immediate nasal allergic response.¹⁰

Bielory et al reported a positive effect of vitamin C in improving white blood cell function and motility with decrease in allergic episodes.¹¹ Anderson et al. stated that ascorbic acid enhances chemotactic function, neutrophil motility and lymphocyte transformation.¹² Kodma et al. found that vitamin C infection or infusion treatment induced an increase of plasma glucocorticoid activity with a delay in onset of about two hours assessed in terms of eosinophil count and plasma cortisol concentration.¹³

The normal range of blood eosinophil count was 4.70 ± 1.26 as percentage of DLC. The normal absolute eosinophil count was $0.3105 \pm 0.7337 \times 10^9/l$. The blood eosinophil counts in allergic rhinitis patients was significantly higher, in the range of $6.38 \pm 3.12\%$ of DLC ($p < 0.01$) to $8.09 \pm 2.091\%$ ($p < 0.05$).

The supplementation with Vit.C dosage significantly decreased the eosinophil counts which approached normal levels. The blood eosinophil count counts after Vit. C treatment was $5.45 \pm 3.56\%$ of DLC and absolute eosinophil counts were $0.386 \pm 0.0313 \times 10^9/l$. The placebo has no significant effect on the blood eosinophil levels. The control mean plasma ascorbic acid levels were 20.45 ± 3.58 mg/l. The plasma ascorbic acid levels in allergic rhinitis patients were 15.45 ± 2.92 mg/l which was significantly lower than the normal values ($p < 0.01$).

Vit. C supplementation increased the plasma ascorbic acid levels to 23.20 ± 5.55 mg/l which were normal levels. Placebo had no significant effect on the plasma ascorbic acid levels.

CONCLUSION

Vitamin C reduces the eosinophilic counts in allergic rhinitis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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