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Original Research Article

Oxidative stress: Can it be a causative factor of defect in calcium Homeostasis in Asthmatic children

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Abstract: Reactive oxygen species induced bronchial abnormalities is an important feature which may leads to asthma specially in children. Vitamin C is an important antioxidant substance known to scavenge the reactive oxygen species. Oxidative injury induces influx dependent changes in the intracellular calcium homeostasis. Antagonism of calcium and magnesium in the asthma and the beneficial effect of magnesium therapy in asthma have also been found in many studies. Children being the major target of asthma, we studied the plasma levels of ascorbic acid, serum ionized calcium, total calcium and magnesium in 50 asthmatic children attending the pediatric OPD and compare with similar number of age and sex matched control. It was found that there was highly significant decrease in the levels of plasma ascorbic acid in asthmatic children (0.43 ± 0.12) as compared to normal control (0.75 ± 0.18). Serum ionized calcium (1.15 ± 0.01 mmol/L), total calcium (8.93 ± 0.96 mg/dl) and serum magnesium (1.91 ± 0.23 mg/dl) in asthmatic children were also significantly decreased as compare to the normal controls (1.24 ± 0.01 mmol/L), (9.35 ± 0.90 mg/dl) and (2.05 ± 0.23 mg/dl) respectively. This significant decrease was irrespective with the age, gender of patients and the severity of disease. In an attempt to scavenge reactive oxygen species, the ascorbic acid is being continuously utilized causing its depletion. At the same time, with less ascorbic acid and continuation of oxidative stress may cause a defect in calcium homeostasis which may lead to increased utilization and translocation i.e. higher influx of calcium ions in bronchial smooth muscle and variety of other cells for e.g. mast cells might have caused its lowering in serum ionized calcium being a physically active fraction which account for nearly half of the total calcium levels. These results suggest that airway hyper-responsiveness may be associated with altered calcium mobilization in airway smooth muscles. Utilization of magnesium for antagonism of calcium in the asthma process might have caused significant lowering in serum magnesium levels in asthmatic children as compare to normal controls.
Keywords:asthma, ascorbic acid, reactive oxygen species, ionized calcium.

INTRODUCTION

Airway inflammation is important characteristics of asthma and the metabolism of oxygen radical is enhanced in symptomatic asthma in relation to clinical disease activity. Eosinophils, alveolar macrophages and neutrophils from asthmatics produce more oxygen species than do those from normal subjects[1]. Ascorbic acid is the most abundant water soluble antioxidant in lung tissue that directly neutralizes free radicals and is a part of Glutathione peroxidase pathway for repairing oxidative damage to the lipid membrane[2]. Ascorbic acid is a potent antioxidant known to protect from oxidative cell death, inhibits Fas ligand induced apoptosis and confers genomic protection through the quenching of intracellular reactive species[3].

In all living being calcium exerts a major role upon diverse physiological processes. Ionic calcium is a physiological active fraction. With respect to asthma its potential role may influence many interrelated processes such as, smooth muscle tone and contraction, mucocilliary function, mast cell mediator release, involvement in inflammatory process, cellular permeability, neurotransmitter function and host of intracellular biochemical events. Its intracellular function is so vital that any drug or agent capable of regulating the entry of calcium into the cell can influence cellular events and metabolism as specific physiological changes[4].

Major element of smooth muscle tension development in asthma is due to an increase in

intracellular calcium concentration after a variety of chemical, electrical or mechanical stimuli has been suggested[5]. There is a possibility of disturbance of calcium homestasis[6] in asthma due to the fact that calcium plays a crucial role in the control of bronchial smooth muscle. Calcium also affects the vascular smooth muscle function[7] and low ionized calcium has been found in the patient of arterial hypertension. In vivo studies have shown that calcium antagonist has both an antihypertensive effect and also an attenuating effect on bronchial hyperreactivity [8]. Low level of ionized calcium has been observed in patients with exercise induced asthma[9].

It has been observed that defect in calcium homeostasis which is called as cellular injury may be due to oxidants. This injury may lead to early and significant increase in ionic calcium in several model of cell injury. Depending on the type of injury, such increase of ionic calcium, were shown to result primarily from influx of extracellular calcium, redistribution of intracellular calcium pool, or both[10].

Several authors have reported a beneficial effect with magnesium therapy in different type of asthma, including the inhibition of bronchoconstriction in asthmatic patients, challenged with a methacholine or histamine bronchoprovocation test[11]. It has been postulated that, the therapeutic effect of magnesium in asthma derives from its action in modulation of smooth muscle contractility and in mediator release through its antagonism of the action of calcium at any one of the several sites [12, 13].

MATERIAL AND METHOD

Present study was carried out in Department of Biochemistry. The permission of the ethical committee was also sought to carry out the present study. Blood samples of 50 (26 males, 24 females) children who came to pediatrics OPD with asthmatic episodes were included in the study. Informed written consent was taken from the parents of the asthmatic children for this study. Blood sample was collected and analyzed for following parameters. Plasma Ascorbic acid was estimated photometrically using Backeman spectrometer[14]. Serum ionized calcium, sodium and potassium were analyzed on ISE based[15] electrolyte analyzer,Easylyte, from Transasia. Total calcium was measured by O cresolphthalein complex one[16] method on semiautomatic analyzer. Serum magnesium[17, 18] was estimated by spectrophotometric method using Randox kit called as magnesium calmagite. Out of total 50 children 18 were having severe symptoms. Equal number of healthy age and sex matched controls (28 males, 22 females) were selected for comparison. All the statistical comparisons were done by using Paired student's t test.

OBSERVATIONS AND RESULTS

In the present study, the result was expressed as mean ± SD in a tabular form. A p value of < 0.05 was considered statistically significant. The total number of 50 asthmatic children was in the age group of 2 to 12 years with similar age matched controls. Plasma ascorbic acid levels in asthmatic children (0.43 ± 0.12) showed highly significant decrease as compared to normal controls (0.75 ± 0.18), serum ionized calcium concentration in the asthmatic children (1.15 ± 0.01mmol/l) also showed highly significant low value as compared to the normal controls (1.24 ± 0.01mmol/l) $P< 0.001$. Total serum calcium and magnesium level in asthmatic children were also significantly low in asthmatic children (8.93 ± 0.96 mg/dl) and (1.91 ± 0.23 mg/dl) respectively as compare to the normal controls (9.35 ± 0.90 mg/dl) and (2.05 ± 0.23) respectively $P< 0.05$. The highly significant low level in asthmatic children was irrespective of gender of subjects and severity of symptoms.

Table 1: Plasma ascorbic acid in asthmatic children in mg/dl

Normal Controls	Asthmatic Children
0.75 ± 0.18	0.42 ± 0.12*

*P<0.001

Table 2: Gender wise distribution of plasm Ascorbic acid in asthmatic children in mg/dl

Gender	Normal Control	Asthmatic Children
Male	0.78 ± 0.21	0.44 ± 0.10*
Female	0.73 ± 0.19	0.42 ± 0.13*

*P<0.001

Table 3: Severity wise distribution of plasma Ascorbic acid in Asthmatic children in mg/dl

Severity	Normal Controls	Asthmatic Children
Mild	0.75 ± 0.18	0.44 ± 0.17*
Sever	0.75 ± 0.18	0.42 ± 0.09*

*p<0.001

Table 4: Serum electrolytes in asthmatic children

Serum Electrolytes	Normal controls	Asthmatic children
Ionized calcium mmol/L	1.24 ± 0.01	1.15 ± 0.01*
Total calcium mg/dl	9.35 ± 0.90	8.93 ± 0.96 l*
Magnesium mg/dl	2.05 ± 0.23	1.91 ± 0.23 l*

*P< 0.05

Table 5: Serum electrolytes and sex variation in asthmatic children

Serum Electrolytes	Normal controls		Asthmatic children	
	Male	Female	Male	Female
Ionized calcium mmol/L	1.24 ± 0.01	1.24 ± 0.02	1.16 ± 0.01*	1.15 ± 0.01*
Total calcium Mg/dl	9.37 ± 0.76	9.34 ± 0.76	8.95 ± 0.82*	8.90 ± 0.80*
Magnesium mg/dl	2.05 ± 0.36	2.04 ± 0.29	1.91 ± 0.21*	1.90 ± 0.19*

*P< 0.05

Table 6: Serum electrolytes according to the severity of asthma

Serum Electrolytes	Normal controls	Severe Asthmatics	Mild asthmatics
Ionized calcium mmol/L	1.24 ± 0.01	1.16 ± 0.02*	1.15 ± 0.01*
Total calcium Mg/dl	9.35 ± 0.90	8.91 ± 0.95*	8.89 ± 0.94*
Magnesium mg/dl	2.05 ± 0.23	1.92 ± 0.14*	1.91 ± 0.21*

*P< 0.05

DISCUSSION

Asthmatics showed increased superoxide generation from leucocytes, as well as increase lipid peroxidation product, indicating increased oxidative stress. Ascorbic acid is an important antioxidant which directly neutralizes free radical, thus it is continuously utilized to maintain the redox state of lungs in asthma[2]. Hatch *et al.;* [19] suggested that Ascorbic acid is the major antioxidant substance present in the airway surface lining of the lung, and may protect against endogenous as well as exogenous oxidants. Our present findings of low Ascorbic acid in asthmatic children could be attributed to its normal physiological function, elevated utilization to overcome continuous generation of oxidants and also to neutralize the exogenous oxidants. Akinkube*et al.;* [20] also reported similar findings who attributed such kind of lowering in ascorbic acid level in plasma to its normal physiological function i.e., its utilization in the maintenance of defense mechanism, tissue integrity and replacement and healing process.

It has been well established that the principle pathogenic features of asthma are ultimately calcium[21] related phenomenon, such as smooth muscle concentration, mast cell chemical mediator secretion, mucous gland secretion and vagal cholinergic reflex activity. More specifically in the cell types, the availability of the free calcium ion for excitation-contraction coupling, stimulus-secretion coupling and nerve impulse conduction, determines significantly the smooth muscle contractility, mast cell mediator secretion, mucous gland secretion and vagus nerve activity etc.

During the course of present study, the mean serum calcium and ionized calcium concentration in the asthmatic children was found to be significantly low as compared to the normal controls. It may be contented that such lowering may be due to the increased infiltration of calcium ions into the tissue cells. This contention gains support from the findings of Kuruda S *et al.;* [22] who confirmed beyond doubt that an elevation in the levels of intracellular calcium in asthmatic adults.

The characteristic features of asthma are due to extreme sensitivity of the airway to physical, chemical and pharmacological stimuli. Exposure of the airway to oxidants, antigen, bacterial infection and environmental pollutants such as fog, smoke may cause a defect in calcium homeostasis[23], leading to increased permeability, translocation and utilization of calcium in a variety of cells such as mast cells, neutrophils and

respiratory smooth muscle cells. Thus with its increased utilization and translocation i.e. higher influx of calcium ions in these cells, might have caused its lowering in plasma. It is also well understood that the ionized calcium is a physiologically active fraction which account for nearly half of the total calcium levels [24]. The mechanism of oxidant induced calcium loading may be due to increased calcium influx through voltage gated calcium channels. This calcium overloads my leads to the induction of contracture[25].

Another important divalent cation, serum magnesium in the asthmatic children was found to be decreased significantly. Similar type of finding has been reported by Rolla G *et al.;* [26] in the individuals with bronchial hyper-reactivity. The reason for such significantly low levels of serum magnesium in asthmatic children is not clearly understood. However, it would be pertinent to mention here that, the role of magnesium as a cation, which has a modulatory effect on the contractile state of smooth muscle cells in the various tissues: Hypomagnesaemia leads to contraction,[27, 28] hypermagnesaemia[29] to relaxation. The relationship between the hypomagnesaemia and increased contractile state may be explained by the antagonism between magnesium and calcium in the cell, and the inhibitory effect of magnesium on the secretion of acetylcholine from the presynaptic neurons[27, 30]. As asthma is characterized by widely varying degrees of contraction of bronchial smooth muscle, magnesium deficiency could have a negative effect on the asthmatic patient, increasing the contracting state of the bronchial smooth muscle. This notion could also be supported by the fact that magnesium infusion was found to improve the pulmonary function in asthmatic adults[12, 31].

CONCLUSION:

In conclusion, it can be suggested that oxidative stress in asthmatic children may cause ascorbic acid depletion which may leads to loss of tissue integrity and ultimately defect in calcium homeostasis. Calcium specially the ionized calcium and serum magnesium levels should be checked in children with bronchial asthma and the low levels should be promptly corrected, not only for its deleterious effect on respiratory muscle power but also for its possible contribution to bronchial hyper-reactivity.

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