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ORIGINAL ARTICLE

AIRWAY ANTIOXIDANT CAPACITY IN ADENOSINE - SIGNALING PULMONARY FIBROSIS IN LUNG DISEASE PATIENT

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Abstract -

Background - Pulmonary fibrosis is a common feature of numerous lung disorders, including interstitial lung diseases, asthma, and chronic obstructive pulmonary disease. Adenosine is a purine-signaling nucleoside that is generated in excess during cellular stress and damage. This signaling molecule has been implicated in the regulation of features of chronic lung disease; however, the impact of adenosine on pulmonary fibrosis is not well understood.

Objective- The goal of this study was to explore the impact of endogenous adenosine elevations on pulmonary fibrosis and to compare ADA activity with total antioxidant capacity in lung disease patients.

Materials and Method- : In the present study 100 healthy non-smokers were served as controls and 60 patients with lung fibrosis were included. Their base line clinical examination Total antioxidant capacity and adenosine deaminase levels were measured. The adenosine deaminase (ADA) activity in the patients at base line was high (P< 0.001) than controls. The total antioxidant capacity

(TAC) was low (P<0.001) in the lung fibrosis patient compared to controls.

Conclusion- These findings demonstrate the adenosine signaling with chronic adenosine elevations was associated with pulmonary fibrosis in lung disease patient with decreased total antioxidant capacity.

Key words- Adenosine deaminase , lung fibrosis, Total antioxidant capacity , Adenosine receptor.

Introduction-

These days due to cigarette smoking, air pollution and other environmental inhalants asthma, chronic bronchitis and emphysema have become rampant and lung fibrosis has risen steadily in incidence. Despite the prevalence of pulmonary fibrosis, the molecular mechanisms governing inflammatory and fibroproliferative aspects of the disorder are not clear. Interstitial lung disease describes a group of heterogeneous lung disorders with variable degrees of pulmonary inflammation and fibrosis. Pulmonary fibrosis is characterized by inflammation, aberrant fibroblast proliferation, and extracellular matrix deposition that results in pathogenic remodeling that eventually distorts pulmonary architecture and compromises pulmonary function [1].

There are many causes of pulmonary fibrosis, including exposure to fibrosis-inducing agents such as silica and coal dust . Pulmonary fibrosis is also a feature found in disorders such as scleroderma sarcoidosis and cystic fibrosis . Idiopathic pulmonary fibrosis (IPF) 3 is a particularly deadly form of pulmonary fibrosis with unknown causes [2]. In addition to these classical forms of pulmonary fibrosis, it is becoming increasingly evident that patients with severe asthma and chronic obstructive pulmonary disease (COPD) also develop features of pulmonary fibrosis [3], which greatly broadens the number of patients afflicted with this disorder. Despite its prevalence, the pathogenesis of pulmonary fibrosis is not completely understood due to a lack of knowledge of the molecular mechanisms governing its onset and progression. Adenosine is a purine-signaling nucleoside that is generated in excess during cellular stress and damage as the result of ATP catabolism.[4]

ADA is an enzyme which contributes in purine metabolism . ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity . Activity of this enzyme increases in lung disease patients [5]

Due to excessive oxidative stress antioxidant imbalance is implicated in the pulmonary fibrosis. Patient oxidant/antioxidant imbalance in favor of oxidants in the airways has been proposed a factor in the pathogenesis of lung disease. [6] Endogenous sources include enzymes which can indirectly produce reactive oxygen species (ROS). The enzyme xanthine oxidase converting xanthine to uric acid also converts oxygen to superoxide anion-radicals during this process & nitric oxide synthase can producenitric oxide radicals which can interact with superoxide anion-radicals resulting in the production of the deleterious oxygen species peroxinitrite Neutrophils may also serve as major contributors of ROS. Following activation these cells undergo a respiratory burst resulting in the release of an eflux of ROS as well as proteinase, cationic proteins & other compounds which may act synergistically to cause oxidative damage in tissues . The living organism has adapted itself to an existence under a continuous efflux of ROS. Among the different adaptive mechanisms the antioxidant defense system is of major importance [7]. This system can be classified into two major groups: the proteins & the low-molecular weight antioxidants (LMWA). The antioxidant proteins contain enzymes (superoxide dismutase, catalase, peroxidase & some supporting enzymes such as glucose-6 phosphate dehydrogenase & glutathione reductase) & the properly proteins (albumin, transferrin, caeruloplasmin & ferritin). The LMWA group contains a large number of compounds capable of preventing oxidative damage by direct & indirect interaction with ROS .

This efficiency of antioxidants is expressed as total antioxidant capacity (TAC) parameter summarizing overall activity of all types of antioxidants in living systems.[8]

Aims & objectives -The goal of this study is to assess the alteration in total antioxidant capacity in adenosine dependent pulmonary fibrosis patients.

In view of the above concepts the present study was undertaken-

1. To make global assessment of antioxidant defenses by measuring total antioxidant capacity in patient with pulmonary fibrosis.
2. To assess lung damage induced alteration in the activity of adenosine deaminase in pulmonary fibrosis.

Materials & Methods -

1. Total antioxidant capacity in plasma (TAC) was assayed by FRAP analysis.[9]
2. Serum adenosine deaminase (ADA) activity was assayed by Giusti G.L. method. [10]

Study design- Distribution of these subjects was as follows.

Sr. No.	Groups	Types	No of Subjects
1	Controls	Healthy subjects	100
2	Patients	Pulmonary fibrosis patient	60

The control subjects were completely healthy non smokers and showed no abnormality on Clinical examination including clinical history and spirometric analysis and were completely symptomless with no history or evidence of atropy or history of asthma . The study was cleared by institutional ethics committee.

10 ml blood sample was collected from each patient 5ml of it was collected in EDTA bulb and 5ml was collected in plain bulb. Plasma and serum were separated from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

The statistical analysis was performed by using student t test and P values < 0.001 were interpreted as statistically significant. The values were expressed as mean $\pm$ SD.

Results -

Table No. 1-Illustrates the levels of Adenosine Deaminase (ADA) and Total Antioxidant

Capacity (TAC) in the healthy controls and pulmonary fibrosis patients.

Sr. No.	Parameters	Types	Pulmonary fibrosis patient n= 60
1	Sr.ADA (U/L)	16.01 ± 1.61	25.23 ± 1.6
2	Plasma TAC(µmol/L)	1263.12± 170.22	354.43 ± 88.22

n = number of cases
All values are expressed in mean ± SD
*** = Significant when compared with control group**

In clinical examination the spirometry analysis of patients in the clinically stable phase of disease with FEV1 / FVC < 70 % were included. In the present study 60 lung fibrosis patients both males or females and 100 controls between the age group of 25-75 years were included.

Discussion-

Table No. 1 displays serum ADA levels in healthy controls and lung fibrosis patients. It was observed that the activity of serum ADA measured in the patients were significantly elevated as compared to healthy controls (ADA) (P<0.001).

Adenosine is a signaling nucleoside that has been suggested to play a role in lung fibrosis through its ability to influence mediator release from mast cells. Adenosine levels are elevated in the lungs of fibrosis patients, further implicating this molecule in the regulation of lung inflammation and suggesting that endogenous increases in adenosine will be useful for the analysis of adenosine function. Adenosine deaminase (ADA), an enzyme responsible for the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine. Adenosine deaminase (ADA) is a purine catabolic enzyme responsible for regulating the levels of adenosine in tissues and cells. [11]

Once produced, adenosine can engage specific G protein- coupled receptors on the surface of cells. Four adenosine receptors (ARs) have been identified (A1AR, A2AAR, A2BAR, and A3AR)[12]. Their expression patterns in tissues and cells are diverse, and their activation can elicit a wide array of cellular responses, including the modulation of inflammatory cell function [13] mast cell degranulation bronchoconstriction [14] apoptosis, cell proliferation and differentiation of lymphocytes, particularly, the

T subtype [15] leading to lung fibrosis.

Table No. 1 shows TAC in healthy controls and patient Significantly diminished TAC activity (P<0.001) was observed in patients with lung fibrosis patients as compared to healthy controls.

Free radical formation is a consequence of a variety of essential biochemical reactions and could be unregulated under pathophysiological conditions. Antioxidants play an important physiological role counteracting free radicals and preventing cellular damage. Lung inflammation is a source of free radical generation. Increased levels of lipid peroxidation products and malondialdehyde [16]. Our study also showed that plasma TAC was significantly decreased in patients with lung fibrosis.

Significant reduction in total ferric reducing ability of plasma may be due to increased free radical activity either because of inflammation or complications that result in imbalance between antioxidant capacity.

ADA has also been considered a marker of cell-mediated immunity. An enhanced ROS generation may promote tissue injury and inflammation. This may further contribute to immunosuppression, and to their impaired antioxidant capacity [17].

Extensively amplified oxidant burden and declined antioxidant levels might be responsible for the observed significant fall in total antioxidant capacity of patients with lung fibrosis.

Conclusion-

Chronic elevations in lung adenosine levels were associated with pulmonary inflammation, expression

of profibrotic molecules, collagen deposition, and extreme alteration in airway structure. These findings demonstrate that chronic adenosine elevations are associated with pulmonary fibrosis and It was hypothesized that pulmonary fibrosis patients have decreased antioxidant capacity in their local airway as compared to the control subject's decline in total antioxidant capacity in their local airway suggest that the adenosine functions as a profibrotic signal in the lung.

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