ASSOCIATION BETWEEN ACTIVITY OF ADAM33 PROTEIN AND OXIDATIVE STRESS IN PATIENTS SUFFERING FROM BRONCHIAL ASTHMA IN JAMMU AND KASHMIR.

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Abstract:

Introduction: Asthma, one of the most common chronic diseases, is characterised by symptoms of random airway blockage and has a range of overlapping phenotypes1. A crucial part of both health and sickness is played by ADAM33, a disintegrin and metalloproteinase glycoprotein engaged in intercellular and cell-matrix interactions.These proteins play a role in myogenesis, neurogenesis, the inflammatory response, and apoptosis in healthy individuals. They do this via altering cell adhesion and cell signalling or through proteolysis. It's significant that the airway epithelium also contains the ADAM33 protein, in addition to the smooth muscle and mesenchymal cells. Oxidative stress may have a role in the aetiology of asthma, a condition that affects the airways and is chronically inflammatory. Acute and chronic inflammation-related features of the airways in asthma include thickening of the airway wall, subepithelial fibrosis, and increased smooth muscle mass. These changes are linked to airway remodelling and may contribute to the development of airflow limitation by increasing airway resistance.

Materials and Methods*:* The study was conducted in 100 patients (70 asthmatic and 30 normal) at GK labs Srinagar Jammu and Kashmir after taking proper consent. BALF was obtained from fibre-optic bronchoscopy. Samples were clarified by centrifugation, and the supernatants were stored at -80°C until required for the measurement of ADAM33 protein activity.

3 mL of blood was collected for the measurement of oxidative stress biomarkers (Lipid peroxides assay, Plasma protein carbonyls).

Results and conclusion*:* In this study, we found that there is an increase in the biomarkers for oxidative stress and activity of ADAM33 protein in asthmatic patients.

Keywords: ADAM33 protein, protein carbonyls, TBARS-MDA, BALF (bronchoalveolar lavage fluid), Oxidative stress

Introduction:

The most prevalent chronic disease, asthma is a respiratory ailment marked by constriction of the breathing passages. It may even pose a life-threatening situation to the patient and have very significant repercussions. (1) The epithelium layer serves as the first line of defence in healthy individuals, trapping allergens in mucus and expelling the mucus and allergens from the airway with the help of cilia. The epithelial layer must keep tight connections in order to carry out this function. These tight connections, however, are broken down in asthmatics, allowing pathogens to enter the body and cause sensitization. Due to this disruption, allergies to the penetrating allergens develop, which causes an inflammatory reaction. Particularly, the

recruitment of eosinophils leads to the production of cytokines, a class of cell signalling proteins involved in immune response, which support the airway inflammation typical of asthma (2). A crucial part of both health and sickness is played by ADAM 33, a disintegrin and metalloproteinase glycoprotein engaged in intercellular and cell-matrix interactions. These proteins play a role in myogenesis, neurogenesis, the inflammatory response, and apoptosis in healthy individuals. They do this via altering cell adhesion and cell signalling or through proteolysis (3-4). It's significant that the airway epithelium also contains the ADAM33 protein, in addition to the smooth muscle and mesenchymal cells. Previous research has shown that airway epithelium does not express ADAM33, although fibroblasts and smooth muscle do.

(5). Human diseases like cancer, heart disease, and immune problems are all influenced significantly by oxidative stress. Oxidative stress may have a role in the aetiology of asthma, a condition that affects the airways and is chronically inflammatory. Recent studies have confirmed the crucial part that oxidative stress plays in the emergence of numerous chronic immunologic illnesses. Traditionally, oxidative stress has been seen as a harmful consequence of aerobic metabolism and a contributor to tissue damage. However, the outcomes of numerous recent investigations have demonstrated that substances linked to oxidative stress, such H_2O_2, function as essential components in signal transduction (6).

Materials and methods:

100 patients (70 asthmatic as test and 30 non-asthmatic as controls) of age 18-85 years were selected on a random basis for the study after taking proper consent from them. The study was done at GK Labs Jammu and Kashmir.

BALF was obtained from healthy controls (n = 30) and patients with asthma (n=70) during fibre-optic bronchoscopy. Samples were clarified by centrifugation, and the supernatants were stored at -80°C until required for the measurement of ADAM33 protein activity.

3 mL of blood was collected in a plain test tube for the measurement of oxidative stress biomarkers, centrifuged and the separated sera was frozen at −20°C until the analyses.

OXIDATIVE STRESS BIOMARKERS

Lipid peroxides assay (for lipid damage)

The phospho-tungstic acid-sulfuric acid combination was used to precipitate plasma lipid peroxides, which were then detected at 532 nm spectrophotometrically. Micro-moles of thiobarbituric acid reactive compounds (TBARS) per litre were used to express the results.

Plasma protein carbonyls (for protein damage)

The content of protein-bound carbonyls in plasma, an indicator of protein oxidation, was measured at 380 nm by using 2,4-dinitrophenylhdrazine according to the method of Levine et al. (8)

Assessment of ADAM33 ENZYME ACTIVITY

A flexible fiberoptic bronchoscope was used to perform bronchoalveolar lavage (BAL) fluid, as described by **Park.et,al** (9)

Processing of BALF

BALF (1 mL) was subjected to Protein A/G pull down using 20 µL Protein A/G PLUS-Agarose to remove Ig from each sample. After adding protease inhibitors with ethylenediamine tetraacetic acid to each sample to a working concentration of 1X, the supernatant (0.15 mg protein/mL) was subjected to Concanavalin A (Con A) pull down using 20 µL Concanavalin A-4B Sepharose beads per mL of BALF supernatant. After pulling down the glycosylated proteins and washing the Con A beads 3X to remove non-specifically bound proteins, the beads were processed for analysis of ADAM33 activity.

Measurement of ADAM33 MP activity

To measure ADAM33 enzyme activity, bound proteins were eluted from the Con A beads using 60 µL of elution buffer (20 mmol/L Tris-HCl, 0.5 mol/L NaCl, 0.5 mol/L methyl a-D mannopyrannoside pH 7.4) and incubated at room temperature for 15 min with intermittent agitation. The beads were removed by centrifugation for 10 min at 4°C before the supernatant was collected for assay of ADAM33 enzyme activity using a fluorescence resonance energy transfer (FRET) peptide cleavage assay,. The assay also included measurement of the activity of different concentrations of recombinant ADAM33 Pro-MP to provide a standard curve that enabled the rates of activity from the BALF samples to be converted into activity units (AU) of ADAM33-like activity per mg of BALF protein .

Results:

Table 1 displays the AU values of the serially diluted samples. When compared to the normal control group, the activity of ADAM33 were considerably greater in the patients with moderate-severe and mild asthma.

Table 1: Correlation between the ADAM33 protein activity (AU) of asthma patients with that of control.

Mild asthma n=28	**Normal control**	**p-value**	**Moderate-severe asthma n=42**	**Normal control**	**p-value**
35± 7.5 AU	5.1± 1.1 AU	<0.0001	298 ±108 AU	5.1± 1.1 AU	<0.0001

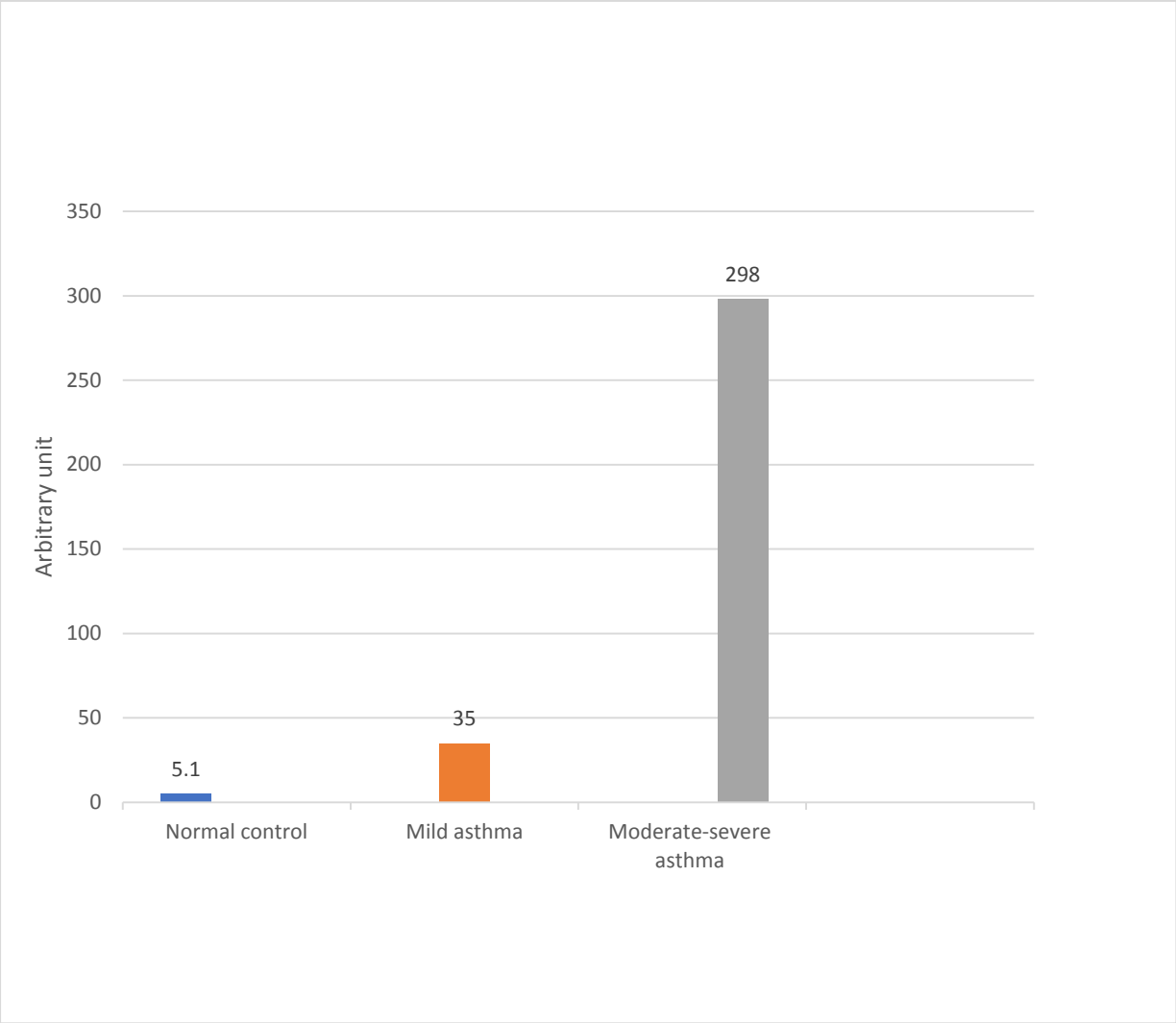

Table 2: Correlation between lipid peroxidation (MDA- TBARS method) and total protein carbonyls in asthmatic and control patients

Study parameter	Normal (30)	Asthmatic (70)	p-value
TBARS ((µmol/L)	2.01±0.2	3.87±0.27	<0.0001
Total protein carbonyls, plasma (nmol/mg protein)	0.9±1.77	2.31 ±0.05	<0.0001

Table 3: Association between activity of ADAM33 protein and oxidative stress biomarkers in asthmatic patients.

Oxidative stress biomarkers (asthmatic patients) n=70		**ADAM33 protein activity (BAL) (asthmatic patient) n=70**	**p-value**	**t-value**
TBARS (μmol/L)	3.87±0.27	298 ±108 AU	<0.0001	22.7
Protein carbonyls, plasma(nmol/mg protein)	2.31 ±0.05	298 ±108 AU	<0.0001	22.9

Discussion:

In the current work, we have demonstrated that, in comparison to healthy participants, patients with asthma exhibit elevated levels of protein carbonyls and lipid peroxidation products, which are indicators of elevated oxidative stress.

For a number of reasons, we decided to investigate the oxidant in the blood sample (plasma) First, the body's blood is a significant reservoir of antioxidant defences(10). Second, asthma is an inflammatory condition in which cells are drawn into the airways from the peripheral circulation. Increased ROS production from the active leukocytes is likely to result in many of the pathologic abnormalities associated with asthma. Third, it is simple to analyse the oxidant using blood. ADAM33 protein activity can be measured from BAL fluid (11).

The increased TBARS and protein carbonyls observed in the present study shows increased oxidative stress in asthmatic patients.

The inflammatory reaction is expected to be sustained and amplified by increased oxidative stress. It's unclear if oxidative stress exposure in the airways can cause airway inflammation. It should be noted that exposure to ozone has been found to cause airway inflammation and hyperreactivity, and that inhaling xanthine-xanthine oxidase has been demonstrated to produce oxygen radicals in vivo, which in turn causes airway hyperreactivity. (12-13)

The ADAM33 protein is expressed in airway smooth muscle cells and fibroblasts, and it has been proposed to contribute to the remodeling process present in asthma. Overproduction or enhanced activity of ADAM33 may lead to excessive shedding of inflammatory mediators, compatible with the enhanced airway wall inflammation present in asthma. Shedding and thereby overproduction of growth factors may furthermore induce proliferation of smooth muscle cells and fibroblasts. These features may lead to the remodeling process present in the airways of asthmatic patients (14)

Many societies have researched the ADAM33 gene's relationship to asthma, and some findings indicate that it may also be linked to lung dysfunction. Because ADAM33 protein plays a "important" part in airway remodelling, it can potentially be thought of as a biomarker of disease severity(15).

Conclusion:

We studied the activity of ADAM33 protein in BAL fluid and oxidative stress biomarker in plasma of blood sample in both asthmatic and control patients. Our study found that there is a

significant increase in the activity of ADAM33 protein as compared to the biomarkers of oxidative stress in asthmatic patients . The result of which are displayed above in the tables.

References:

1. Full Report of Expert Panel: Guidelines for the Diagnosis and Management of Asthma.2007.
2. Kudo, M., Ishigatsubo, Y., & Aoki, I. (2013). Pathology of asthma. Frontiers in Microbiology, 4, 263-263.
3. Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL, et al. Association of a disintegrin and metalloprotease 33 (*ADAM33*) gene with asthma in ethnically diverse populations. *J Allergy Clin Immunol.* 2003;112:717–22.
4. Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal.* 2006;8:243–270.
5. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the *ADAM33* gene with asthma and bronchial hyperresponsiveness. *Nature.* 2002;418:426–30. [PubMed] [Google Scholar] [Ref list]
6. Fratta Pasini A., Albiero A., Stranieri C., Cominacini M., Pasini A., Mozzini C., Vallerio P., Cominacini L., Garbin U. Serum oxidative stress-induced repression of Nrf2 and GSH depletion: A mechanism potentially involved in endothelial dysfunction of young smokers. *PLoS ONE.* 2012;7:e30291. doi: 10.1371/journal.pone.0030291.
7. National Institutes of Health. Expert panel report on guidelines for diagnosis and management of asthma. Bethesda, Md: National Heart, Lung, and Blood Institute Information Center; 1997.
8. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. In: Packer L, editor. Methods in enzymology. Vol. 186. New York: Academic Press; 1990. p. 464-72.
9. Park CS, Chung SW, Ki SY, Lim GI, Uh ST, Kim YH, Choi DI, Park JS, Lee DW, Kitaichi M. Increased levels of interleukin-6 are associated with lymphocytosis in bronchoalveolar lavage fluids of idiopathic nonspecific interstitial pneumonia. Am J Respir Crit Care Med 2000; 162:1162–1168
10. Van Asbeck BS Hoidal J Vercelloti GM Schwartz BA Moldow CF Jacob HS Protection against lethal hyperoxia by tracheal insufflation of erythrocytes: role of red cell glutathione. *Science.* 1985; 227: 756-759
11. Cluzel M Damon M Chanaz P Bonsquet J Crastesde Paulet A Michel FB et al. Enhanced alveolar cell luminol- dependent chemiluminiscence in asthma. *J Allergy Clin Immunol.* 1987; 80: 195-201
12. Holtzman MJ, Fabbri LM, O'Byrne PM. Importance of airway inflammation for hyperresponsiveness induced by ozone. Am Rev Respir Dis 1983;127:686-90
13. Katsumata MJ, Miura M, Ichinose M, Kimura K, Takahasi T, Inoue H, et al. Oxygen radicals produce airway constriction and hyperresponsiveness in anaesthetized cats. Am Rev Respir Dis 1990;141:1158-61.
14. Wei Lin Xue, Wei Han, Zhao-Shan Zhou. ADAM33 polymorphisms are associated with asthma and distinctive palm dermatoglyphic pattern. Mol Med Rep.2013; 8(6):1795-1800.
15. Shweta Sinha1, Jagtar Singh1, Surinder KJ. Significant Association of ADAM33 V4C>G Polymorphisms with Asthma in a North Indian Population. Bioresearch Communications.2015; Volume 1, Issue 2. July.