ISSN: 0975-3583,0976-2833 VOL14, ISSUE 02, 2023

Correlation of antioxidants and IgE levels and its comparison with ADAM33 gene polymorphism (V4) in asthma patients of Jammu and Kashmir.

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Abstract:

Introduction*:** Asthma is a severe breathing disorder which effects the bronchial airways and makes it difficult to breath out. Many factors like increase in biochemical markers, environmental factors and genetic polymorphism can be associated with the onset of Asthma. The aim of this study is to determine whether IgE and antioxidant levels are associated with genetic polymorphisms of the *ADAM33* gene (V4) in both healthy and asthmatic patients. ***Materials and methods: The study was conducted in 100 patients (70 asthmatic and 30 normal) at GK labs Srinagar J&K India. ***Results*:** In this study, we found that there is a significant correlation between IgE and antioxidant levels in asthma patients compared to controls in population of Jammu and Kashmir where there was an increase in IgE level and decrease in antioxidant level in addition to ADAM33(V4) gene polymorphism.

Keywords: ADAM33 gene polymorphism, SNP V4, IgE, Glutathione, Albumin.

Introduction:

Asthma being one of the most prevalent chronic diseases has a number of overlapping phenotypes (1) and it is defined by symptoms of sporadic airway obstruction. It is a long term respiratory disorder which is characterized by variable and reappearing symptoms and bronchospasm (2). In asthma, the airways exhibit acute and chronic inflammation-related characteristics which are linked to airway remodelling, including thickening of the airway wall, subepithelial fibrosis, and increased smooth muscle mass, which may aid in the development of airflow limitation by raising airway resistance.

Some of the symptoms of asthma are excessive coughing, tightness of the chest and breathlessness that may occur a few times a day or a few times a week (3). Genetic factors like polymorphisms, family history and environmental factors (tobacco smoke, wood dust, etc) can contribute to the development of asthma (4). A subset of the zinc-dependent metalloproteinase that produces surface proteins with adhesion and protease activity is A Disintegrin and Metalloprotease 33 (*ADAM33*) gene which is located on 20p13 of human chromosome. It is one of the members of ADAM family that plays an important biological role as an activator of growth factors and Th2 cytokines (5). ADAM gene proteins play an important role in cell proliferation, differentiation, migration, and embryogenesis (6).

Antioxidant glutathione shields tissues and cells from oxidation. As a result, the lungs' airways contain an antioxidant mechanism that shields them from contact with damaging oxidants Severe asthma lowers glutathione levels, which causes airways to overreact (7). Low glutathione levels increase oxidative stress because they reduce muscle contraction. There is evidence that allergy conditions such allergic rhinitis, allergic skin dermatitis, and bronchial asthma generate oxidative stress [8].

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IgE, a glycoprotein with a high concentration in asthmatic patients while having a normal level of (0.001%) of the total blood immunoglobulin, is characterised by its high concentration (9). In the immunopathogenesis of asthma, IgE is crucial. An indication of allergic inflammation atopy and a sign of illness progression is an elevated IgE content. Patients with asthma who have greater quantities of IgE are more prone to develop allergies (10).

There is a strong correlation between ADAM33 and asthma, where it has been shown to be highly expressed in fibroblasts of the airways and brain tissues. The smooth cells in the lungs' airways become dysfunctional due to ADAM33 polymorphism, and the lining of the lungs deteriorates [11]

Materials and methods:

100 subjects aged between 18-85 years were selected for the study in which 70 subjects were asthmatic and referred to as test and 30 normal subjects were used as controls. The study was done at GK Lab Srinagar (J&K) India. Proper consent was taken from the subjects before collecting the samples.

Sample Collection:

Each sample was split into two portions under strictly aseptic circumstances. The first portion (2 ml) was collected in an EDTA tube for DNA extraction. The second portion of the blood (3 ml) was collected in a simple test tube for the measurement of biochemical tests, centrifuged, and the separated sera were frozen at 20°C. The blood was centrifuged for 5 minutes at 4000 rpm to separate the plasma, which was stored at -80°C. To prevent inaccurate findings from many freeze/thaw cycles, samples were well mixed and thawed soon before the experiment. Hemolyzed and lipemic samples were also avoided.

Measurements of immunological and antioxidant parameters:

Serum IgE levels was measured using Enzyme linked immunosorbent assay (ELISA). Glutathione, total protein and albumin were measured by using spectrophotometer for all asthmatic patients and control**.**

DNA extraction and genotyping of ADAM33 (V4):

Genome DNA extraction

Each individual had 2 mL of peripheral venous blood drawn from them and sent for EDTA-based EDTA anticoagulation. DNA was extracted from whole blood using a whole blood genome DNA extraction kit (TIANGEN, DP348-02), and it was then kept at -80˚ C.

Primer design

Primer Premier 5 software was used to create the primers in accordance with the gene sequence provided by GenBank. On the basis of SNaPshot quality control over double-blind samples and a negative control for the classification outcomes, classification was carried out.

PCR amplification

The PCR reaction system totaled 20μL, which included: genome DNA 1 μL, Ex taq Mix 10 μL, upstream primer 1 μL, downstream primer 1 μL, and ddH2O 7 μL. PCR reaction conditions: 5 min pre-denaturation under 95˚C, 20 s denaturation under 95˚C, 20 s annealing under 60–68˚C, 30 s extension under 72˚C, with 35 cycles in total, 5 min terminal extension

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under 72˚C. PCR product was tested using 2% agarose gel electrophoresis, and DNA sequencing was performed.

RFLP detection

ADAM33(V4) polymorphism was determined by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR - RFLP) technique.

The primers used for PCR-RFLP are:

Forward primer	5'-ACA CAC AGA ATG GGG GAG AG-3'
Reverse primer	5'-CCA GAA GCA AAG GTC ACA CA-3'

The Pst I restriction enzymes was used for digestiom detection of the V4 loci. Enzyme containing amplified PCR reaction product 10 µL, restriction enzyme 0.5 µL, ddH2O 7.5 µL, and 10 x NE Buffer fluid 2 µL. It was placed at a temperature of 37˚C for one hour and 65˚C for 20min. 3% Sepharose gel was used for electrophoresis of the enzyme-digested product for 30 min and voltage maintained at 120 V. After gel imaging, Quantity One 1-D software was used to perform image analysis. The digestion product were visualized on 3% agarose gel electrophoresis stained with Redsave in the presence of 50 bp DNA ladder (Biolabs) as a molecular marker.

Three types of genotypes were shown, which is single fragment (374 bp) as a CC homozygous, 2 fragments (204 and 169 bp) as a GG homozygous and 3 fragments (374, 204, 169 bp) as a GC heterozygous.

All the statistical data was calculated with the help of SPSS 16 version 2

Results:

The comparison of IgE and antioxidant levels of the asthmatic patient and control groups participate in this study are presented in table 1:

Parameter	**Groups**		**P-value**
	Test (70)	**Control (30)**	
IgE	(210.12±21.5)	(57.2±3.97)	< 0.0001
Glutathione	(9.8± 1.81)	(15.93± 4.17)	< 0.0001
Albumin	(4.16± 1.02)	(5.20± 0.60)	< 0.0001

Table 1

The results shown in the table above indicates that there is a significant increases in the IgE levels in asthma patients as compared to control group, while there were a significant decrease in the levels of antioxidants (glutathione and albumin) of the asthma patients compared to the control groups.

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Comparison between IgE levels and ADAM33 gene polymorphism (V4) in asthmatic patients and control group is shown in table 2

SNP name	**Genotypes**	**IgE levels**		**p-value of test**	**p-value of control**
		Test(70)	Control (30)		
V4	CC	138.7 ± 7.1 (42)	16.2 ± 1.5 (18)	CC vs CG P = 0.0608	CC vs CG P = 0.9116
	CG	135.1 ± 6.1 (19)	16.1 ± 3.1 (8)	CC vs GG P = 0.0016	CC vs GG P = 0.9394
	GG	130.4 ± 4.8 (9)	16.3 ± 4.9(4)	CG vs GG P = 0.0531	CG vs GG P = 0.9394

Table 2

The association of IgE levels and ADAM33 gene (V4) did not show any significant relation in asthmatic patients and healthy control.

Discussion:

The association of IgE levels with polymorphisms of the *ADAM33* gene specifically V4 SNPs was shown in this study conducted among asthmatic patients of Jammu and Kashmir where we found that there is an increase in IgE levels in asthmatic patients. IgE is an antibody produced by the immune system in response to allergens that the body may be exposed to. The ADAM33 protein is expressed in airway smooth muscle cells and fibroblasts, and it has been proposed to contribute to the remodelling process present in asthma (12). It is unknown whether the production or activity of ADAM33 in asthma is increased or reduced. 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 remodelling process present in the airways of asthmatic patients. However, the present results have suggested that this may also reflect a general phenomenon because polymorphisms in ADAM33 could be associated with acceleration in the decline in the ventilatory functions in subjects exposed to Asthma dust containing allergic agents.

Conclusion:

In conclusion, our results demonstrate that there was a significant correlation between elevated IgE and decreased antioxidant levels in addition to ADAM33(V4) gene polymorphism were associated with asthma patients compared to controls in population of Jammu and Kashmir. The present study focused on SNP (rs2787094/V4) of ADAM33 gene, detection of ADAM33 (V4) SNP was successfully amplified and the results showed this SNP was associated with asthma, these results are consistent with previous results in Iraqi population (13)

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