Original Article

ADAM33: Role and pathogenesis study in COPD in Delhi NCR Population

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is caused by various environmental influences and genetic determinants. ADAM33, (Disintegrin, Metalloproteinase 33) is identified as one of the candidate gene playing an important role in airway pulmonary disease. Various studies have been conducted to reveal the genetic polymorphism of ADAM33. Our objective was to identify the role of ADAM33 in the blood serum samples of patients and its correlation with the FEV/FVC. The serum levels of ADAM33 from the blood samples were studied to correlate with the severity of the disease. The ADAM33 Single Nucleotide polymorphism was also studied to evaluate their association with COPD in Population of Delhi-NCR. The study was conducted on 100 patients at Rajan Babu Institute for Pulmonary Medicine & Tuberculosis (RJPIMT) Hospital. Patient diagnosis of COPD was confirmed by Spirometric test. Our result reveals a strong negative correlation was seen between FVC/FEV and ADAM33 for smoker group whereas weak correlation was seen between FEV/FVC and ADAM33 in non-smoker group of COPD patients. It also showed S1AA, ST+5AA were significantly more frequent in cases than in controls (P < 0.001). However T1, V4, ST+5 genotypes were found to be expressed more in the controls.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is known as one of the common and complex pulmonary disease which is identified as crucial hazardous diseases across globe. The disease has caused an extensive increase in morbidity and mortality rate of life all across the world. It is among one of the disease-causing large amounts of economic burden over the globe. WHO reports states that COPD will be the fourth largest leading cause of death by 2020[1] Under the stated guidelines of Global Initiatives for Chronic Obstructive Lung disease (GOLD), COPD is defined as one systematic common preventable, treatable but not curable disease which is characterized by persistent airflow progressive limitation and associated with inflammatory responses in the airway to noxious particles or gases. Another variable of exacerbation and comorbidities contributes towards the severity of diseases [2]. Pathologically COPD consists of two major conditions of Emphysema; causing shortness of breath due to over-inflammation of the alveoli saccs[3] and Chronic Bronchitis; production of a chronic cough and sputum for at least three months a year for two consecutive years [4]. COPD patients show airway hyperresponsiveness (AHR) to non-specific stimuli in airway obstruction. The severity of AHR is directly correlated with inflammation in lung tissues and increased number of CD8 cells in bronchial epithelium of COPD lungs [5] The most important cause for COPD is particulate -mater and cigarette smoking affecting lungs [6] Upto 25-42% of COPD patients with chronic limitation of airflow are non-smoker [7] It is well observed and documented that non-smoking related factors (genetic or exposure to air pollution) may be another vital causative agents for Chronic Obstructive Pulmonary Disease [8]. In stable COPD patients increased levels of cytokines such as IL-6, IL-8, TNF-α are seen in playing key role in pathogenesis of diseases.

Since all the COPD patients are not smoker or are not long term tobacco consumer there are many other significant contributing factors which plays role in progression and development of COPD [9]. Environmental and Genetic interactions play an important role in the development of COPD [10]. Individuals with tobacco exposure show greater relativity in terms of development of respiratory disease. It is well suggested that a genetic factor might be responsible in the progression and development of COPD in the...
non-smoker patients [\ldots]. The candidates genes which are identified playing a significant role in protease-antiprotease pathway, oxidant-antioxidant pathways and inflammatory responses are studied as a potential gene for the genetic association in COPD [\ldots]. In particular for the genesis of pulmonary emphysema, proteases in extracellular matrix (ECM) and Metalloproteinase (MMP) plays a significant role in parallel to inflammatory cytokines [\ldots]. As revealed in the study conducted by Van Erdewegh and coworkers disintegrin and Metalloprotease 33 (ADAM33) gene is identified as most susceptible gene for asthma and Bronchial hyperresponsiveness [\ldots]. ADAM33 is known as a transmembrane metalloproteinase from a subgroup of zinc-dependent superfamily of more than 30 members [\ldots]. ADAM33 expresses its significant role in lung fibroblasts, bronchial muscles and it is involved in processes of cell adhesion, proteolysis and cell signaling by releasing many factors [\ldots] and developing key role in pulmonary defense mechanism and tissue remodeling. In chronic obstructive pulmonary disease the only biomarkers used is lung function testing based on Forced expiratory volume (FEV). But unfortunately FEV ratio does not reproduce accurate and correct underlying respiratory disease. Need for developing a novel biomarker is to identify its role in the pathogenesis of disease. In many ongoing studies none of the revealed markers for the disease fulfilled all the requisite of biomarkers. It is well observed that additional biomarkers are needed for better sensitivity and specificity for the prognosis of COPD [\ldots]. In the progression of lung disease blood samples may be a convenient source to be used as a biomarker. The blood biomarker plays a significant role via releasing from the lungs into the bloodstream, which reflects active and progressive state of disease.

SNPs contribute in the transcription and expression of mRNA and Proteins in ADAM33 [\ldots]. The expressed proteins are further responsible for pathogenesis of AHR in ADAM33. Various studies have also revealed that polymorphism in ADAM33 has lead to airway hyper-responsiveness and inflammation in COPD and is also responsible for decline in lung function [\ldots]. With decline in Lung functioning; FEV/FVC, increasing mortality and morbidity risk of COPD, and increase in patients of smoker and non-smoker group it becomes important to study its genetic aspects of the gene ADAM33 which increases the susceptibility to COPD.

The present study was conducted with the background, to ascertain the relationship between lung functioning FEV/FVC and ADAM33 in various COPD and control groups. The study also relates the role of inflammation and its effects on susceptible gene expression by correlating TNF-\(\alpha\) and ADAM33 in the population of Delhi-NCR.

The study simultaneously tries to study the single nucleotide polymorphism of gene ADAM33 which might give an insight in the genetic study of the disease and help to explore better novel biomarker and healthcare treatment for the disease in near future.

**Materials and Methodology:**

For the detailed study and detection of the severity of disease complete and comprehensive medical history is required. The differential overlapping diagnosis diseases for COPD are asthma, congestive heart failure, bronchiectasis, Tuberculosis, obliterative bronchiolitis and pulmonary fibrosis. Patients detailed information of observed symptoms, medical history, family history, previous exacerbation or any other respiratory disorders [\ldots] are collected through structured and validated questionnaire.

The study was carried out in chest OPD on patients attending the pulmonary medicine department of Rajan Babu Institute for Pulmonary Medicine & Tuberculosis (RJPIMT) Hospital Hospital Hospital (RJPIMT), GTB Nagar, New Delhi. The study was conducted after the ethical clearance from the review board of Hospital (SNo 0591/ RBIPMT/2016). The patients from various age groups were included in the study which was conducted on 100 patients suffering from COPD with or without smoking as a prime factor. The patient Blood samples were collected only after informed consent from each subject included in the study.

**Inclusion Criteria:** All patients who are diagnosed with COPD after spirometry confirmation of FEV/FVC < 70% and post-bronchodilator FEV/FVC<80% as defined by the GOLD guidelines. Various symptoms are also added on as a key indicator helping in better accessibility of disease. A fully informed written consent with the detailed clinical history was collected from the subjects. All inclusion and exclusion criteria were fulfilled.

**Exclusion Criteria:** Patients, with Sputum positive pulmonary tuberculosis, asthma patients and patients with bronchiectasis, acute exacerbation or with any other comorbidity like cardiovascular, diabetic or any other disease are excluded from the study.

**Spirometry:**

For confirmatory diagnosis of chronic obstructive pulmonary disease, GOLD recommended the spirometry test. The spirometry test is a technique and a qualitative analysis of post-bronchodilator FEV/FVC (Forced expiration volume in 1st sec/ Forced vital capacity). Along with this, GOLD also suggested some indicator for the better detection of disease. The primarily exist are: age of an individual should be more than 40 years, dyspnea, which goes characteristically worsen with exercise, chronic Cough and Sputum production. The presence of such indicator increases the probability of disease Spirometry includes test conducted. Further guidelines for standardized lung function test were adopted for the present study. Information was clearly directed to the patient prior conducting spirometry.
### Table 1: The characteristics of Smokers and Non-Smoker group:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smoker</th>
<th>Non-Smoker</th>
<th>Smoker</th>
<th>Non-Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.25(10.47)</td>
<td>54.86(10.01)</td>
<td>50.66(9.71)</td>
<td>50.39(9.47)</td>
</tr>
<tr>
<td>Male</td>
<td>100%</td>
<td>78.30%</td>
<td>100%</td>
<td>78%</td>
</tr>
<tr>
<td>Female</td>
<td>NA</td>
<td>21.60%</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>Pack years</td>
<td>30.16</td>
<td>NA</td>
<td>12.18</td>
<td>NA</td>
</tr>
<tr>
<td>PEV/FVC</td>
<td>47%</td>
<td>52%</td>
<td>83%</td>
<td>85%</td>
</tr>
</tbody>
</table>

### RESULTS:

#### Statistical Analysis:

Data are expressed as mean ±S.D. The comparison of variables between smokers and non-smokers COPD and smoker and non-smoker control were performed using students T-Test. As Pearson p value below 0.05 was considered statistically significant. The Pearson's correlation was used to evaluate the relationship between variables.

The ADAM33 serum levels were significantly higher in the smoker group of COPD patients compared to the non smoker group.

### Serum ADAM33 Estimation:

Serum ADAM33 assay was conducted using Human ADAM33 (A Disintegrin and Metalloprotease 33) ELISA Kit. It is an in vitro enzyme-linked immunosorbent sandwich ELISA assay for the quantitative estimation of human ADAM33 in serum. This assay employs an antibody specific for human ADAM33 which is coated on a well plate.

Samples and standards were pipetted into the well plate. ADAM33 present in the sample binds to the well by immobilized specified Ab in the incubation period. Wells were then washed and biotinylated detection antibodies specific for Human ADAM33 are added to each micro plate well successively and incubated. After washing the unbound antibody the HRP-Conjugated Streptavidin was added to the well and colour was developed in amount of the TNF-α bounded. Stop solution was added to stop the reaction which changed the colour from yellow to blue and then plate was read in ELISA reader at the 450 nm.

### DNA Extraction & Genotyping:

4 ml peripheral blood samples were collected from each subject for DNA isolation. Plasma was separated after centrifugation and genomic DNA was extracted using Qiagen DNA whole blood extraction kit.

After the extraction of genomic DNA its purity was checked by Nanodrop, Nanovalue plus Spectrophotometer model, GE Healthcare. Nanodrop purity check is to calculate the A260/A280 ratio in order to look for protein contaminations. A good quality DNA sample should have a A260/A280 ratio of 1.7 - 2.0 and we have got the A260/A280 ratio equal to and above 1.8 for all the samples.

The isolated DNA sample was quantified by using NANO Drop Assay and qualitified by DNA gel running respectively. The DNA sample was stored at -20°C. The Genetic analysis of five different single nucleotide polymorphism for the gene ADAM33 (T1, T2, S1, ST+4, ST+5) were screened and performed. These SNPs were found relatively to be significantly associated with excessive decline in lung function and in COPD []. Amplification of gene was carried out in a gradient PCR thermal cycler from Bio-Rad (India) Pvt. Ltd. Amplification were carried out in 25µl volume as followed in Table 2.
of COPD patients (P<0.05). It was also identified that a strong negative correlation was seen between FVC/FEV and ADAM33 for smoker group whereas weak correlation was seen between FEV/FVC and ADAM33 in non-smoker group of COPD patients as represented in Graph 1. The variables of the four study group are shown in Table 1.

It was also observed that the average age is not statistically significant between the smoker and non-smoker group of control and diseased patients. The result reveals that the serum level of ADAM33 was significantly elevated in smoker group of COPD than smoker group of control patients.

There is a significant correlation (P<0.001) between Smoker group of COPD and Smoker group of Control Group for ADAM33 shown in Figure 1. The serum level of ADAM33 between diseased smoker and Non-smoker group of COPD was statistically significant (p< 0.005) as shown in Figure 2.

The genetic analysis was done for five single nucleotide polymorphism of ADAM33 gene T1/Rs(rs2280091),T2/Rs(rs2280090)/S1/Rs(rs612709),ST+4/Rs(rs44707), ST+5/Rs(rs59798). These SNPs were relatively found out to be associated with the excessive lung function and pulmonary disbalance. The DNA samples were amplified using the hotstart master mix and polymerase chain reaction was performed with different PCR Condition using Icycler Thermal Cycler.

The study consists of 120 COPD patients and 100 Control samples. The mean age of the patient was 54.25 and control sample was 50.66 respectively. Male population consisted 100% for smoker and 78% for control population respectively.

Comparing the genotype and allele frequencies of control and COPD patients, the S1AA, ST+5AA were significantly more frequent in cases than in controls (P < 0.001). However T1,V4, ST+4 genotypes were found to be expressed more in the controls.

The frequencies of alleles, S1G and ST+5 G was higher in the control group than in the patient group, whereas the frequencies of allele A was higher in T1, V4, ST+4 in patient group than control group. The data for RFLP genotyping was analyzed using the appropriate statistical tool via SPSS. The data was described and intra-group comparison was done by parametric/Non-parametric test. A P value of less than 0.05 was significantly considered.
Agarose gel electrophoresis for RFLP–PCR product of ADAM33 T1 stained with ethidium bromide. 1st and last column – represents Ladder. Lanes 3, 6, 9, 11, 12 represent heterozygote AG genotype. Lanes 4, 5, 8, 10 homozygote AA genotype.

Table 4: Association between Allele frequency and COPD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases N= 120 (%)</th>
<th>Control N=100 (%)</th>
<th>P value OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 SNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>45</td>
<td>42</td>
<td>0.65</td>
</tr>
<tr>
<td>AG</td>
<td>59</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>V4 SNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>39</td>
<td>46</td>
<td>0.065</td>
</tr>
<tr>
<td>AG</td>
<td>54</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>28</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>S1 SNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>50</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>32</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>38</td>
<td>32</td>
<td>0.001</td>
</tr>
<tr>
<td>ST+4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>38</td>
<td>35</td>
<td>0.881</td>
</tr>
<tr>
<td>AG</td>
<td>60</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>22</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>ST+5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>53</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>44</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>24</td>
<td>20</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

Agarose gel electrophoresis for RFLP–PCR product of ADAM33 S1 stained with ethidium bromide. 1st column and last column – represents Ladder. Lanes 2, 4 represent homozygote AA genotype. Lanes 3, 9, 10 and 7 represent homozygote GG genotype. Genotype Lanes 5, 6, 7, 8 represent heterozygote AG genotype.

Agarose gel electrophoresis for RFLP–PCR product of ADAM33 S1 stained with ethidium bromide. Last column – represents Ladder. Lanes 2 represent homozygote AA genotype. Lanes 10 represent heterozygote AG genotype. Lanes 4, 5, 8, 9 represent homozygote GG genotype.
DISCUSSION:

COPD is a progressive disease with limited airflow in the lungs due to chronic bronchitis and emphysema which leads to the destruction in alveoli and pulmonary disease. To validate the role of genetic association of gene ADAM33 various SNPs T1, V4, S1, ST+4, ST+5 were genotyped and analyzed in Indian Delhi-NCR population by means of case-control approach.

ADAM proteins are Zn2+ dependent metalloproteinase involved in signal transduction. Van Eerdewegh et al. (Nature 2002) revealed ADAM33 as a susceptible gene for asthma; He stated that ADAM33 is expressed in airway smooth muscle cells and lung fibroblast and plays significant role in airway remodeling because of its high expression in epithelium, myofibroblasts or fibroblasts and airway smooth muscle cells (ASMCs)[]. Over or under expression of ADAM33 leads in alterations of airway remodeling and repair processes[]. In the above said view the present case-
control study focus on the polymorphism in SNPs of ADAM33 and their role in pathogenesis of COPD. The present case–control study is the demonstration of an association between ADAM33 polymorphism and COPD in the Delhi-NCR population. The sample size of our study was 220 out of which 120 were patient and 100 were healthy control sample size. Majority of our sample size population accounts for 93.6% as males. The study aims to identify the role of ADAM33 in the pathogenesis of COPD. Levels of ADAM33 in blood were found to be strongly correlated with the diseased or control group. High level of ADAM33 was found in serum samples of Smoker / Non smoker group. To the best of our knowledge, this is one of the first study conducted in Delhi-NCR population which showcase a clear strong correlation between decreased FEV1/FVC and elevated serum ADAM33 level in the blood samples of COPD patients.

The present case–control study is the first demonstration of an association between ADAM33 SNP polymorphisms and COPD in the Delhi-NCR Population of India. In this association we genotyped 120 well characterized COPD patients and 100 well healthy control samples for five SNPs (T1, V4, S1, ST+4, ST+5) of the ADAM33 gene. The analysis was done through the PCR-RFLP technique and revealed that the SNPs were significantly associated with COPD patients.

In 2002, Van Erdeweigh and co-workers showed the ADAM33 (a disintegrin and metalloprotease 33) gene polymorphism as a suspected gene for bronchial responsiveness[31] Many studies have also revealed the association of ADAM33 with asthma in different population [3]. It is also observed that the association between allele of ADAM33 polymorphism is associated with an excessive decline in lung function.

Study conducted by Jongepier et al. over 200 asthmatic patients found that SNP allele of S2, T1 and T2 of ADAM33 were associated with an excessive decline in FEV1[3]. Simpson et al. in his cohort study conducted on European population also revealed an association between F+1, M+1, T1 and T2 of ADAM33 with impaired lung function[3]. Many other studies were conducted all across the world which shows the association of SNPs T1, ST+5, T2, Q1, S1, S2, V1 and V4 of ADAM33 gene with reduced lung function[3].

Studies have shown a relationship between ADAM33 gene variation and COPD. Van Diemen and his colleagues in his study revealed that in COPD subjects for homozygous minor allele of SNP S2 and Q1 and heterozygous SNP s1 have an excessive decline in FEV1 compared to the wild type[3].

Gosman in his study found that ADAM33 SNPS (ST+5, T1, T2, and S2) are associated with the pathophysiology of COPD in airway hyper-responsiveness, inflammatory cells and CD8 in bronchial biopsies[3]. A case control study in the Caucasian population demonstrated the association of SNPS (Q-1, S1, S2, V1, V4) with COPD and lung function decline in long term smokers[3]. Similar finding were also revealed by Wang et al. in his study conducted on Chinese Han population for four SNPs (T2G, T1G, S2C, and Q-1G alleles (P<0.001).

Jun-Lung Xiao et al. in his study conducted on Tibetan Population said long term smoking COPD patients have relative association with four SNPs (V4, T2, T1, and S1)[3]. Many other studies have also shown no association between the SNPs T1, T2 of ADAM33 as COPD.

In our study we observed that the homozygous wild allele AA genotype of T1, S1, ST+4, ST+5 and is higher in patients than the control pointing a significant role in development of COPD.

CONCLUSION:

Our study revealed a significant association between elevated level of ADAM33 in the serum samples of the COPD population as well as it reveals significant genetic association between SNPs of ADAM33 gene in the studied Delhi-NCR Population. Various studies have revealed a strong relationship between asthama/COPD with ADAM33 SNPs. It is very evident that there are some common SNPs of ADAM33 gene which accounts for pathogenesis of both the diseases, suggesting an accelerated dysfunction of lung in disease progression. However some specific SNPs accounts for specific disease. At present the mechanistic roles of the disease-associated SNPs have yet to be elucidated, especially in the context of the pathophysiology of asthma and COPD.

Since we have included limited number of SNPs from ADAM33 gene to the restricted population sample size there is still scope for more candidate SNPs in the potential gene ADAM33 which implicates genesis in the COPD population.

References