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Orignal Article

Association of ADAM33 SNP (RS528557) Gene Polymorphism with COPD In Pakistani Population

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a major health Problem worldwide. It is currently the fourth leading cause of death with the highest morbidity and mortality throughout the world. ADAM33 has been implicated in the etiology of asthma, another obstructive pulmonary disease. **Objective:** Research shows that its genetic polymorphism may play a pivotal role in COPD pathophysiology; however, data is still inconclusive and no research has been done on it in Pakistan. **Methods:** A total of 102 subjects (51 cases + 51 controls) were recruited. Blood samples were drawn for deoxyribonucleic acid (DNA) isolation from individuals. DNA extraction and Polymerase Chain Reaction (PCR) was optimized and restriction fragment length polymorphism (RFLP) was carried out by incubation at 37 C with digesting enzyme' FseI' for minor allele rs528557. Data was analyzed by using SPSS version 26.0. Data for age, pack smoking/year, frequency of exacerbation and BMI was described by mean ± SD. Alleles and genotypes were described as proportions and percentages. Comparison of the said variables between two groups was performed by Chi-Square as applicable. **Results:** G allele was found in all cases (100%) and in 74.5% controls at p= <0.001. On the other hand, the frequency of minor allele C was 11.8% and 29.4% in cases and controls respectively at p=0.03. Homozygous major genotype (G/G) was 88.2%, in controls versus 70.6% in cases (p=0.09).Heterozygous genotype (G/C) was 9.2% in controls and 25.5% in cases. Similarly homozygous minor genotype (C/C) was 0.8% in controls and 3.9% in cases respectively at p=0.56. **conclusion:** Thus, we show that G allele of rs528557 may be associated with risk of COPD in Pakistani subjects.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a devastating disease which has affected millions of people worldwide. It is the third leading cause of death and frequently occurs with comorbidities [1-3]. It is characterized by inexorable small airways obstructive deterioration and emphysema associated with structural remodeling and cellular inflammation along with squamous and epithelial metaplasia leading to fibrosis [4]. COPD is thought to occur due to the slow, progressive and cumulative inflammatory response to inhaled particles, primarily from cigarette smoke and biomass fuel pollution. Till now, the mechanism of COPD is still unknown, however, life style modification and advancement in medical sciences may able to enhance life expectancy [5]. Smoking is the most significant cause of COPD. Smoking causes unusual inflammatory lungs changes that lead to airflow obstruction [6] Other causer includes various genetic and environmental factors [7]. The symptoms of the disease include permanent airflow obstructions and invasion of certain neutrophils, macrophages, and CD8 lymphocytes in the airways of lungs. Many of the individuals suffering from COPD have airway hyper- responsiveness (AHR). COPD is a multifactorial process comprising of cell profiles such as neutrophils, lymphocytes eosinophils and monocytes, in addition to higher levels of cytokines such as interleukin 6 (IL-6) and interleukin 8 (IL-8).[8]. TNF-α and VEGF also play

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an important role in COPD individuals [9]. The variation in an individual's life expectancy is due to a family of protein called ADAM33 (a Disintegrin and Metalloproteinase), a membrane bounded protein family. One of the members of ADAM family, A single nucleotide polymorphism in this 'disintegrin and metalloprotease, ADAM33, discovered in 2002, is related to the development and progression of COPD [10,11]. A research has proved that SNPs located in the ADAM33 locus, influenced lung function [12] and were found to be linked with COPD [13]. The ADAM33 gene contains 179 single nucleotide polymorphisms loci (SNP). However, S2 is the most studied SNP [14]. SNP's have also been researched to be connected with BHR and have been seen to decrease FEVI in patients who have COPD [15]. The polymorphism of the S2 locus present in the ADAM33 has been linked to the development of COPD. To the best of our knowledge S2 SNP has never been studied in the Pakistani population in any disease including COPD. We therefore designed a case control study to investigate the association of this SNP with risk of COPD in local population.

METHODS

Data collection and sampling method: The study was endorsed by The Ethical committee of IMBB, University of Lahore. Indoor/outdoor patients satisfying the inclusion/exclusion criteria were asked for their written informed consent for interest for participation in research project. Inclusion criteria included all diagnosed smokers of COPD with evidence from spirometry who has more than 25 pack years of smoking history. Our determined sample size was calculated to be 10 with 95% confidence level and 99% power of study using the study of Laxmi e. al(2016)[16]. However, for better power of study we took 110 subjects with 51 for each group. Non-probability, convenient sampling was used.Smokers, both men and women, with age of 40 years or more, were selected.Controls and cases were matched for age, gender and years of smoking. Controls were asymptomatic for chronic respiratory illness. Detailed history and physical examination were performed and patient pro forma were filled. Height and weight were measured and BMI was determined as follows: BMI=(weight in kg)/m2. 5 ml blood sampleswere drawn from antecubital vein by aseptic technique in EDTA vacutainers. Fresh spirometry was not done due to restrictions of COVID pandemic and previous records of pulmonary lung function were used to confirm diagnosis of COPD as per international criteria [17].

PCR_RFLP: DNA was extracted using commercial kit by following manufacturer's instructions. 0.8% gel was used for DNA isolation along with 1kb DNA ladder. The gel from electrophoresis was stained in a solution containing ethidium bromide and visualized in a gel documentation system under UV illumination. The S2 region was amplified for cloning with the help of polymerase chain reaction(PCR) kit Thermoscientific PCR master. The primersweredesignedtopartiallyamplifytheS2sequence.The primers sequences were as follows: R, 5'-AGAGCTCTGAGGAGGGGAAC3':F,5'TGTGCAGGCTGAAAGTATGC -3' (Laxmi et al., 2016). The final product of 304 base pairs was confirmed through Gel electrophoresis. After PCR all PCR products were digested overnight at 37oC with restriction enzyme "FseI" according to the manufacturer'sprotocol.2%gelwaspreparedfor thisstepasmentionedabove.Thentheproductwasanalyzed by agarose gel electrophoresis and visualized under UV illumination.

StatisticalAnalysis: All data were analyzed using SPSS for windows version 26. Significance was set at $p<0.05$. Continuous variables were expressed as mean±SD while Qualitative variables were expressed as proportions and percentages. Difference in distribution of genotypes and alleles of the SNP rs528557 between controls and COPD was tested with Chi-square. An online calculator www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-alleles.html was used to detect any significant deviations from Hardy Weinberg equilibrium. Genotype distributions were compared between cases and controls by Chi square. $P\leq0.5$ was considered statistically

RESULTS

Characteristics of Subjects: The demographic and clinical data is shown in table 1

CHARACTERISTIC	COPD n=51	Controls n=51	P value
Age years (mean±SD)	57.71+12.87	52.08+9.03	0.01*
Gender n (%)	Males= 41 Females=10	Males=24 Females=27	<0.001**
Pack years of smoking	22.15±1.68	4.50±8.77	<0.001**
BMI KG/M2 (mean±SD)	31.26±9.08.01	35.35±8.19	0.02*
Patient with chronic cough n (%)	51(100)	47(92)	0.04*
Smoker/non-smokern(%)	51/0 (100/0)	11/40 (21.6/78.4)	<0.001**
Breathlessnessn(%)	49 (96)	41(80)	0.01*
Any cardiac diseasen(%)	4 (8)	2(4)	0.40
Diabetes mellitusn(%)	12(24)	18(35)	0.19
Hypertensionn(%)	11(22)	3(6)	0.02*
Osteoporosisn(%)	0(0)	6(12)	0.01*
Clinical anxiety or depressionn(%)	2(4)	3(6)	0.64
Regularsputumproductionn(%)	46(90)	29(57)	<0.001**
Frequency of G allelen(%)	51(100)	38(74.5)	<0.001**
Frequency of C allelen(%)	6(11.8)	15(29.4)	0.03*
Frequency of G/G genotype n(%)	45(88.2)	36(70.6)	0.08[s]
Frequency of G/C genotype n(%)	5(9.2)	13(25.5)	
Frequency of C/C genotype n(%)	1(0.8)	2(3.9)	

Table 1: Comparison of study variables between COPD cases and

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controls Significant p≤0.05, ** highly significant p<0.001**, ap value by Chi square test

Detection of SNPS in Cases and COPD Patients: For the SNP under study, rs528557, the 304 base pair product was restricted into three bands of54, 42 and 19 base pairs for genotype G/G genotype, 96bp and 19bp forC/Cgenotype, and all four bands (96, 54, 42, and 19 base pairs) for the heterozygous genotype G/C. Purified PCR products of the S2 primer pair (350bp) were digested by the enzyme FseI. The genotype distribution among cases was in accordance with Hardy Weinberg equilibrium(p=0.56) but the controls near to deviate significantly from HWE at p=0.09. As shown in table 1, the genotype G/G of SNP rs528557 is over-represented in cases (88.2% versus 70.6%) while C/G and C/C are overrepresented amongst controls (3.9% vs 0.8%). However, Chi square test showed that this distribution failed to reach statistical significance (p=0.08). Allele frequency distribution showed significant results with G allele dominant in COPD (p<0.001) and C dominant in controls(p=0.03).

DISCUSSION

There are two significant findings of our study. First is the association of 'G' allele with risk of COPD. Second is the identification of comorbidities of COPD as shown in table 1. The current study investigated the association of human ADAM33 gene Polymorphism with COPD in the Pakistani population. ADAM33 is a zinc-dependent metall oproteinase that regulates its own activity as well as the activity of a wide variety of other proteins through proteolytic cleavage. A total of seven domains are present in ADAM33, including a pro-domain, a catalytic domain, a metalloprotease domain, a disintegrin domain (which interacts with integrins), a cysteine-rich/epidermal growth factor domain (which promotes cell-cell interaction), a transmembrane domain, and a cytoplasmic domain. There are also several splice variants containing different combinations of these domains [18]. ADAM 33 is prominently expressed in smooth muscle cells, fibroblasts and myofibroblasts, hence by, is an important protein for the proper functionality of different human organs including lungs. Various previous studies have associated the polymorphism in ADAM33 with risk of COPD.[19-22] Diemen et al. (2005) showed this association in a Dutch general population study in which more than a thousandparticipantsweregenotypedforeightADAM33SNPs. The present study has demonstrated a higher frequency of SNP S2 major allele 'G' in patients of COPD as compared to healthy controls. This is similar with the findings of Sadeghnejad et al. (2009) and Wang et al. (2009). Figarska et al. (2013) also studied ADAM33 gene polymorphisms including S2 and showed that COPD patients with ADMA33 polymorphism (including SNPs Q-1, S1, S2, and T2) had a higher mortality than others. This could be explained by the physiology of ADAM33 protein. Excessive expression of ADAM33 may lead to the release of inflammatory mediators and growth factors which in turn may induce pathological changes like the proliferation of smooth muscle cells and fibroblasts. Dysfunction of fibroblasts and smooth muscles is part of the pathophysiology of COPD [23]. Thus, ADAM33 polymorphism can put a smoker at risk to develop COPD and make a COPD patient susceptible to complications, leading to increased morbidity and mortality [24,25]. As to the mechanism, howrs528557 is linked to pathophysiology of COPD, it maybe explained as follows. S2 along with S1is located in exon 19of ADAM33 which encodes the transmembrane region of the protein. S2 is a silent SNP, not associated with any amino acid substitutions. Thus, it might not directly cause structural change in ADAM33.However, this non-coding SNP may disturb the splicing process, indirectly affecting protein structure. Alternatively, S2 may be in linkage disequilibrium (LD) with another closely situated SNPs, possibly S1, which might actually be responsible for the functional change in ADAM33 [26]. The present study also described the COPD association with lower body weight, undernutrition due to chronic inflammatory processes, dyspnea, diabetes mellitus, hypertension, cardiac illness and osteoporosis and found that except DM and cardiac disease, hypertension, lower body weight, dyspnea and osteoporosis are significantly associated risk factors of COPD.The presence of comorbidities by gender and GOLD stage were also presented by Van Eerdewegh et al. (2002). Other reported risk factor associated with COPD include smoking, aging, lack of physical inactivity downregulates the anabolic states that result in diminished testosterone levels, reduced bone density and mass of muscle [27]. The osteoporosis in COPD is contributed potentially due to inflammation, corticosteroid usage, anemia, smoking and hypogonadism [28, 29]. The hypertension and COPD is linked to the aging factor that leads to loss in connective tissues and increases the stiffness of arteries that may pre dispose the systematic hypertension in COPD patients [30].

CONCLUSION

There are several limitations to this study. While we uncovered a statistically significant association of the 'G' allele with risk of COPD, the genotype frequency association was not statistically significant. This might be attributed to a modest sample size. Second, due to COVID precautions we could not record bronchial hyper reactivity in COPD patients as we would have liked, since BHR is strongly correlated with COPD in previous studies. Finally,

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inclusion of other SNPs especially S1 could have further enhanced the findings of our study and future studies should proceed in that direction. Despite the above limitations we believe this study provides useful information about another candidate SNP to the accumulating data of genetic polymorphism that make smokers susceptible to COPD. Thus, patients at risk of COPD may be identified in future.

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