POLYMORPHIC VARIANTS IN *SOD-1* AND *CATALASE* GENES AND ITS RELATION TOWARD MODULATING THE RISK IN COPD

SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS

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IN

BIOTECHNOLOGY

By

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DECLARATION

I hereby declare that the work done in this dissertation report entitled, "Polymorphic variants in *SOD1* and *Catalase* genes and its relation towards modulating the risk in COPD" submitted towards partial fulfillment requirement for the award of **Master of Science degree** in Biotechnology in **The Department of Biotechnology** of **Thapar Institute of Engineering and Technology, Patiala** is an authentic record of work carried out by me under the supervision and guidance of **Dr. Siddharth Sharma, Associate Professor** of **Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala.**

This matter embodied in this report has not been submitted in part or whole to any other university or institute for the award of any degree.

Tathagata Pal.

DATE: 14th July, 2023.

TATHAGATA PAL

This is to certify that the above declaration made by the student concerned is correct to the best of my knowledge and belief.

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CERTIFICATE

This is to certify that the dissertation entitled "Polymorphic variants in *SOD1* and *Catalase* genes and its relation towards modulating the risk in COPD" submitted by **Mr. Tathagata Pal** in partial fulfillment of the requirements for the award of M.Sc. in Biotechnology at Thapar Institute of Engineering and Technology, Patiala, is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in this dissertation has not been submitted to any other university/institute for the award of any Degree or Diploma.

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ABSTRACT

Chronic obstructive pulmonary disease (COPD)'s etiology has been linked to increased oxidative stress. This study looked at the cytosine to thymidine transition at nucleotide -262 (-262 C>T) of the catalase gene, the 50-bps insertion-deletion polymorphism at 1684 bp upstream of the ATG start codon of Copper-Zinc superoxide dismutase (Cu Zn-SOD), and their relation towards modulation the risk of COPD. The participants were healthy controls and stable COPD patients (n=200, for both cases and controls), matched for their average age. The catalase gene at -262 C>T and Cu Zn-SOD 50 bp Ins/Del were genotyped, and statistical analysis was performed to check for the risk factors associated with the disease.

Del/ Del genotype was found in 5.5% of the subjects, and T/T was found in 10.0% of the subjects, which were significantly higher than controls, 1.0%, and 2.5% in both cases, respectively. A significant association of risk factors was for both genes, suggesting their association in modulating the risk of the disease. In the case of Cu Zn-SOD, a significant six-fold increase was observed in the co-dominant model for the Del/Del genotype (AOR=6.56, 95% CI=1.35-31.88, p=0.0197). While in the case of catalase, a significant approximate increase of five-folds was observed in the co-dominant model for T/T genotype (AOR=4.62, 95% CI=1.63-13.11, p=0.003). A significant association of risk was also found to be linked with the patients' CAT score, age, and GOLD group. A combinational study of the polymorphism involving both genes obtained a significant increase in the associated risk with the increased alternations, where a total of three alterations in both the genes combined were linked with a more than seven-fold risk in the patients (AOR=7.71, 95%CI=1.58-37.5, p=0.0114).

KEYWORDS: Chronic Obstructive Pulmonary Disease, Single Nucleotide Polymorphism, Catalase, Copper Zinc Superoxide dismutase

TABLE OF CONTENTS

ABBREVIATIONS USED

%	Percentage
µg	Microgram
mg	Milligram
g	Grams
µL	Microliter
mL	Milliliter
°C	Degree Celsius
COPD	Chronic Obstructive Pulmonary Disease
GMC	Government Medical College, Patiala
AAT	α-1 Antitrypsin
ECM	Extracellular matrix
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic acid
SNP	Single nucleotide polymorphism
WHO	World Health Organization
CDC	Center for Disease Control
NCDC	National Center for Disease Control
FVC	Forced Vital Capacity
FEV1	Forced Expiratory Volume
IL	Interleukin
GRO-a	Growth related oncogenes-a
GM-CSF	Granulocyte monocyte colony stimulation factor
TNF	Tumor necrosis factor
PAMPs	Pathogen associated molecular patterns
PRRs	Pattern recognition receptors
NLRs	Nucleotide-binding domain leucine-rich repeat-containing receptors
TLRs	Toll-like receptor
DAMPs	Damage associated molecular patters
HMGB1	High mobility group box 1
BAL	Bronchoalveolar Lavage
MMP	Matrix Metalloproteinase
ATP	Adenosine tri-phosphate
RAGE	Receptor for advanced glycation end products
Nrf2	Nuclear erythroid-2-related factor-2 (Nrf2)
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
CAT	Catalase
EC-SOD	Extracellular superoxide dismutase
PCR	Polymerase Chain Reaction
EC	Enzyme Commission
CAT score	COPD Assessment Test
NADP	Nicotinamide adenine dinucleotide phosphate

GOLD	Gold Initiative for Lungs Disease
ATS	The American Thoracic Society
ERS	European Respiratory Society
LLN	Lower limit of normal
mMRC	modified Medical Research Council
EDTA	Ethylenediamine tetra acetic acid.
SDS	Sodium Dodecyl Sulfate
RCF	Relative Centrifugal Force
PCI	Phenol Chloroform Isoamyl alcohol
UV	Ultraviolet
RNA	Ribonucleic Acid
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
BSA	Bovine serum albumin
$MgCl_2$	Magnesium Chloride
dNTPs	deoxy-Nucleotide tri-phosphate
RFLP	Restriction Fragment Length Polymorphism
RE	Restriction Endonuclease
SD	Standard deviation
OR	Odds Ratio
CI	Confidence interval
AOR	Adjusted odds ratio

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogenous term for progressive lung diseases. It is a group of lung diseases where there is an obstruction in airflow in the lungs. Progressive lung diseases get worse over time, meaning less airflow in and out of the lungs (Gibson *et al.,* 2009). That means there is not sufficient oxygen provided to tissues/cells in the lungs as well as in the body. Cigarette smoking is the leading cause of COPD. Though, it is not the only cause. Cooking in closed non-ventilated kitchens and air pollution can also cause COPD. A rare genetic disorder, where there is a deficiency of alpha-1 antitrypsin (AAT), can also be the cause for the same. AAT is a protein synthesized in the liver that maintains the balance of protease and anti-protease in the lungs. Due to the deficiency, the balance is disrupted, and ECM and tissue degradation occur, especially in the lungs. Another key characteristic feature of COPD is that the patient has Forced expiratory volume, which means the volume of air a person can forcefully exhale in 't' seconds, progressively lowers (Wu *et al.*, 2014)

Almost all patients with COPD suffer from two main conditions: Emphysema and Chronic Bronchitis.

- *Emphysema*: Emphysema is a condition that affects the air spaces distal to the terminal bronchiole. Its characteristic features are the permanent enlargement of lung air spaces with the destruction of their walls without fibrosis and the destruction of lung parenchyma with loss of elasticity. Small perforations are generated in lung tubes. (Gibson *et al.,* 2009).
  Emphysema is generally caused by chronic and significant exposure to cigarette smoke, smoke in general and noxious pollutants.
- *Chronic Bronchitis*: Inflammation associated with bronchi is termed bronchitis. Due to inflammation, hyper mucus production takes place. There are different types of bronchitis. But the most common are acute and chronic. A long and persistent type of inflammation is called chronic inflammation. It is common among smokers. Patients with chronic bronchitis are susceptible to lung infections more easily. Exacerbations of high acute inflammation are also common among patients. (Gibson *et al.,* 2009).

Reactive oxygen species (ROS) are generated when a person inhales smoke, whether from cigarette smoking or air pollution. ROS is harmful and, thus, causes mutations in DNA. ROS can oxidize nitrogenous bases in the DNA. For example, when ROS oxidizes guanine, 8-oxo-guanine is formed, which can now bind to adenine and thymine (Rom *et al.*, 2013).

Due to mutations, cells undergo apoptosis. This leads to the generation of damage-associated molecular patterns (DAMPs). DAMPs can bind to various receptors on inflammatory cells, especially activating neutrophils and macrophages. Toll-like receptor-4 concentration was found to be elevated in COPD patients. Thus, high amounts of cytokines are produced, increasing the influx of more immune cells. Activated neutrophils further produce more ROS, triggering the pathway even more. This leads to lung tissue damage. (MacNee, 2007). Antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase neutralize ROS, thus limiting the damage. ROS are also known to activate proteinases in the lungs, which cause tissue damage. So, it is crucial to maintain the concentration of ROS. Polymorphic variants of these enzymes and proteinases compromise the balance of ROS because these mutations change the enzyme conformation and activity (MacNee, 2007).

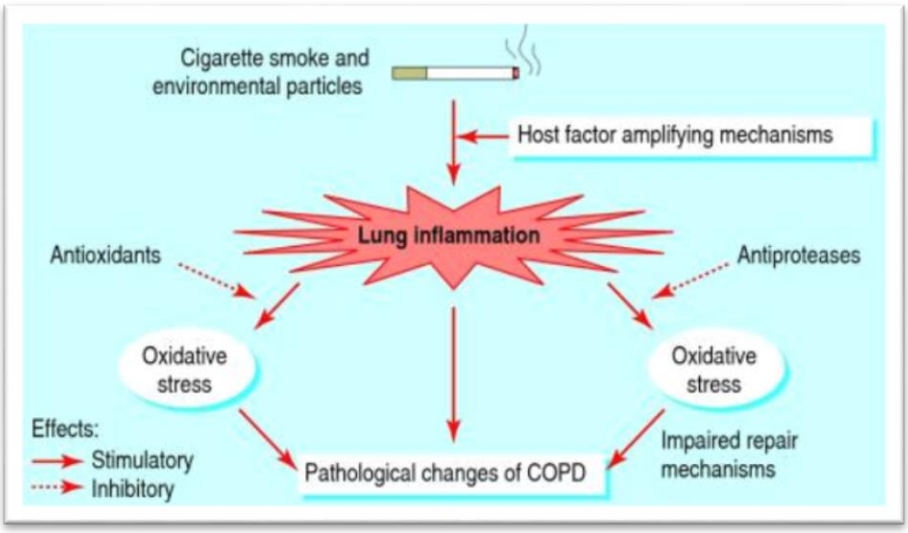


Fig 1: The pathogenesis of COPD (McNee, W 2006).

Single nucleotide polymorphism (SNP) is a type of genetic variation where a single nucleotide in the DNA sequence is mutated. When about 1% of the population carries a different nucleotide at a particular position, it is called an SNP. If the SNP exists in a gene, then the gene is termed to have different alleles. SNPs may lead to variation in amino acid in the protein, but SNPs present in the non-coding region generally alter the transcription rate (Brooks *et al.*, 1999).

Almost all enzymes are proteins, so their structure is essential for their specific and proper catalysis. This again holds for antioxidant enzymes also. Alteration of the nucleotide sequence in gene and non-coding segments of DNA such as promoters, terminators, enhancers, etc., determining the transcription rate affects enzyme availability and catalysis. Mutations in these antioxidant enzyme genes affect the catalytic activity of these enzymes due to impaired protein structure; thus, both cases affect the disease severity due to lower enzyme availability, ROS present in the cells, and intercellular spaces are not neutralized (Crawford *et al.*, 2012).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is a hypernym used for heterogeneous lungs condition characterized by various abnormalities in airway passage (bronchitis) and in alveoli (emphysema) leading to various chronic respiratory symptoms such as cough, dyspnea, mucus production, breathlessness, etc. The primary cause of COPD is the inhaling of noxious particles and gas. It is a progressive disorder destroying lung parenchyma, causing inflammation, alveolar attachment loss, mucus obstruction and limited lung airflow. (Celli *et al.*, 2022). Lung elasticity is also hampered in COPD due to the high tissue remodelling rate. For over 200 years, COPD has remained one of the primary lung diseases claiming the highest morbidity and fatality worldwide (Warren *et al.*, 2009).

2.2 Epidemiology of COPD

2.2.1 Prevalence and Mortality Worldwide

COPD is the third leading cause of death worldwide, claiming about 3.23 million deaths in 2019. About 90% of deaths were associated with lower-income and middle-income countries. In the case of higher-income countries, 70% of the total cases are caused due to tobacco smoking. The same risk factor of smoking drops 30-40% of cases in lower- and middle-income countries. Household air pollution is another significant risk factor in lower- and middle-income countries. COPD is the seventh leading cause of poor health worldwide (WHO, 2023).

In 2017, COPD accounted for 544 million cases worldwide, among which 55.1% were men and 54.8% were women. In 2015, the total number of cases was below 174 million, causing 3.2 million deaths worldwide. The prevalence of COPD was higher by about 20% in adults over 40. (Szalontai *et al.*, 2021)

2.2.2 Prevalence and Mortality in India

Crude estimates suggest that there are about 30 million COPD patients in India. COPD mortality has been increasing alarmingly over the years, and India is considered to have one of the highest death rates due to COPD in the world. In 2016, COPD cases in India were a staggering 55.3 million, the second leading cause of death due to non-communicable disease. Over the years, studies have concluded that the prevalence of COPD increased after 30 years of age. The estimated prevalence of COPD ranged from 0.1% to 0.9% between the age group of 5 years to 29 years, while the incidence ranged from 1.6% to 28.3% in the population above 30 years of age. The widespread presence of the disease varies across the region and states. According to the data from NCDC, the prevalence of COPD in Delhi was 10%, while in Kerala, it was 6.19% and 4.36% in Bangalore. Gender-wise variation in the prevalence of COPD rates in males ranged between 2% to 22% and that for females between 1.2 to 19% (Verma *et al.,* 2021)**.**

2.2.3 Economic burden

COPD is linked with a significant economic burden. In the U.S., the cost allotted for treating and preventing COPD is estimated to increase alarmingly in the next 20 years. Experts have predicted the projected cost to rise to 40 billion dollars annually. Out of the 6% direct cost of the total respiratory disease healthcare budget, the European Union spares 56% of the 6% for COPD alone. There is no doubt that the treatment cost is directly proportional to the severity of the disease. (Zafari *et al.,* 2021) In ordinary to intensive care, the average hospital stay for COPD varies from 4.5 to 16 days (Dalal *et al.,* 2011). In 2010, the direct cost of treating COPD patients in developed countries such as the United States was $36 billion, and 16.4 million days of work were lost due to COPD each year, adding a significant burden to the person and population level (CDC,2014).

In the case of lower- and middle-income countries, the disadvantage plays a combinatorial effect due to both smoking and poor environmental conditions. As the health care budget is already low, the higher cost of inhaled medicine for COPD is unavailable in these countries reported by WHO. This situation is similar in the case of spirometry availability. This, in turn, can create a domino effect on the patient's family; due to the worsening health, a family member has to leave their job to care for their needs (Stolbrink *et al.,* 2022).

2.3 Pathogenesis of COPD

In the early days, COPD was found to be only affected by environmental factors. But research revealed that COPD results from a complex interaction of genetic and environmental factors, damaging or altering lung structure and functions over the lifetime. To understand the interaction of these factors, the branch of GETomics was proposed. GETomics proposes the complex interaction of genetic and environmental factors concerning time (over the ageing process) (Agusti *et al.,* 2022).

2.3.1 Smoking and passive smoking

It has been evident that cigarette smoking is the critical environmental factor responsible for the pathogenesis of the disease. Almost 50% of the smoker's population develops COPD and a gradual decrease in lung function, thus hampering the FEV1 value. The mortality rate is higher than that of non-smokers, and it is estimated that at least 50% of all COPD cases worldwide result from smoking. Mutations and genetic factors can worsen the process in the smoker population. The rate of pathogenesis is different among gender. Social factors, such as peer pressure, play an important role. A person as a passive smoker is always at higher risk as the inhalation of noxious particles, and gases is significantly higher than absolute non-smokers. Smoking during pregnancy affects the lung growth and development of the foetus, thus further increasing the risk of the disease. Not only tobacco smoking, marijuana smoking, such as pipes, cigars, water pipes etc., plays an equal role in the disease pathogenesis (Bårdsen *et al.,* 2022).

2.3.2 Biomass exposure

Tobacco smoking remains the leading risk factor in the case of high-income and developed countries, contributing to approximately 70% of the total cases. However, as about 85% of the total cases are contributed by lower- and middle-income countries, there remains a significant gap in the risk factors involving smoking and other environmental factors. Only 30-40% of the cases registered in these countries are due to tobacco smoking (Yang *et al.,* 2022). Wood, animal dung, crop residues, and coal, typically burned in open fires or poorly functioning stoves, may lead to very high levels of household air pollution. One of the significant risk factors for females in these countries is cooking in a non-ventilated kitchen. Over 3 billion people worldwide use biomass and coal as their primary energy source for daily and household needs, thus putting them at a greater

risk. Non-smoker COPD is more common in females and young individuals showing similar respiratory abnormalities and quality of life, lesser neutrophil concentration in the airway passage while higher eosinophil concentration, similar macrophage abnormality of phagocytosis, lesser levels of emphysema but higher small airway obstruction and similar spirometry values (Salvi et., 2020).

2.3.3 Air pollution

In absolute non-smokers, air pollution is the leading cause of COPD. Particulate matter, sulphur and nitrogen oxides, greenhouse gases and heavy metals pose a risk of approximately 50% in lower- and middle-income countries. The pathogenesis of COPD due to air pollution is totally dose and type of pollutant dependent without any safe threshold. (Murray *et al.,* 2020)

2.3.4 Genetic causes

A significant hierarchical risk of COPD has been found in smokers suggesting genetic involvement.

- *Alpha-1 antitrypsin (AAT) deficiency*: AAT deficiency is a rare genetic disorder that causes COPD. The lung's function depends on elastin fibres surrounding the airway and alveolar walls. An enzyme called elastase, present in normal lungs and upregulated in smokers' lungs, can damage the airways and alveoli by forming perforations. AAT, produced in the liver and transported through the blood, is an anti-protease that checks elastase activity. The PIZZ genotype of AAT is responsible for decreased protein in the body. However, the PIM3 allele, considered to be expected, was reported to be associated with COPD patients in the Indian population (Gupta *et al.,* 2005)
- *Serpine2:* Serpine 2 had been a significant tissue and cell-associated inhibitor of thrombin and plasmin, but not elastase. It has been considered necessary due to its expression pattern and relationship with alpha-1 antitrypsin. It was shown that specific lung cells express Serpine2, and its expression was altered in individuals with specific clinical characteristics of COPD. (DeMeo *et al.,* 2006)

2.3.5 Occupational hazard

This is the most under-appreciated environmental risk factor of COPD. Individuals exposed to higher doses of inorganic dust, organic fumes, chemical agents and pesticides pose a much higher risk of developing respiratory obstruction, respiratory symptoms, and emphysema and gas trapping in both men and women. The risk of occupational studies is considered much higher in the non-regulated areas of the world, as most of these studies have been conducted in North America and Europe (Paulin *et al.*, 2006).

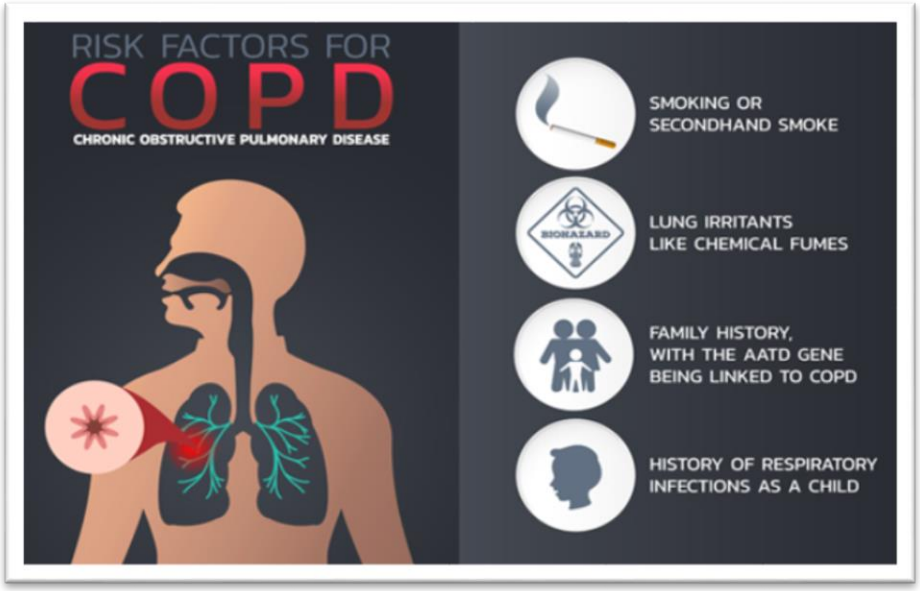


Fig 2: Risk factors in the development of COPD (**Source**: https://www.medicoverhospitals.in/diseases/copd/)

2.4 Symptoms of COPD

- Shortness of breath, especially with physical activity.
- An ongoing cough or a cough that produces a large amount of mucus is often called a smoker's cough.
- Chest tightness
- Wheezing- a whistling or squeaky sound while breathing
- Frequent lung infections
- Weight loss and tiredness

2.5 Pathophysiology

Pathophysiology, in other words, explains disorders body's physiological processes associated with the disease; for patients with COPD, irreversible lung damage is associated with lung parenchyma and airway damage, thus, resulting in breathlessness, mucus and chronic cough.

2.5.1 Airflow Obstruction and gas trapping

Due to parenchymal destruction and small airway disease, airflow obstruction is caused in COPD patients. The degree of obstruction varies from person to person. Due to chronic inflammation, the narrowing of small airways and destruction of lungs parenchyma results in loss of alveolar attachment, leading to decreased lung capacity of gaseous exchange. This limits the ability to expand of lungs. All these sum up only to one fact of lungs losing the forced expiration capacity causing FEV1 and FEV1/FVC ratio to drop and contributing to gas trapping (Hogg *et al.,* 2004)

2.5.2 Pulmonary gas exchange abnormalities

Structural abnormalities in alveoli, small airway changes and changes in pulmonary circulation in COPD patients are the primary reasons for abnormal gas exchange. This further leads to atrial hypoxemia varying in severity from person to person. Lung defusing capacity is decreased due to emphysema caused by parenchymal destruction (Elbehairy *et al.,* 2015)

2.5.3 Exacerbations

Exacerbations or worsening of disease with triggered symptoms in COPD patients can happen for multiple reasons, including a coexisting infection of virus or bacteria, sudden inflation of high environmental pollutants, etc (Parker *et al.,* 2005). During exacerbations, there is hyper-inflammation in the lungs, increased gas trapping and reduced expiratory flow, causing the patient to suffer from high breathlessness. This might also trigger hypoxic conditions. Frequent exacerbations can be associated with the severity of the disease, making the patient highly susceptible to comorbidities (Barberà *et al.,* 1997, Rebe *et al.,* 2007).

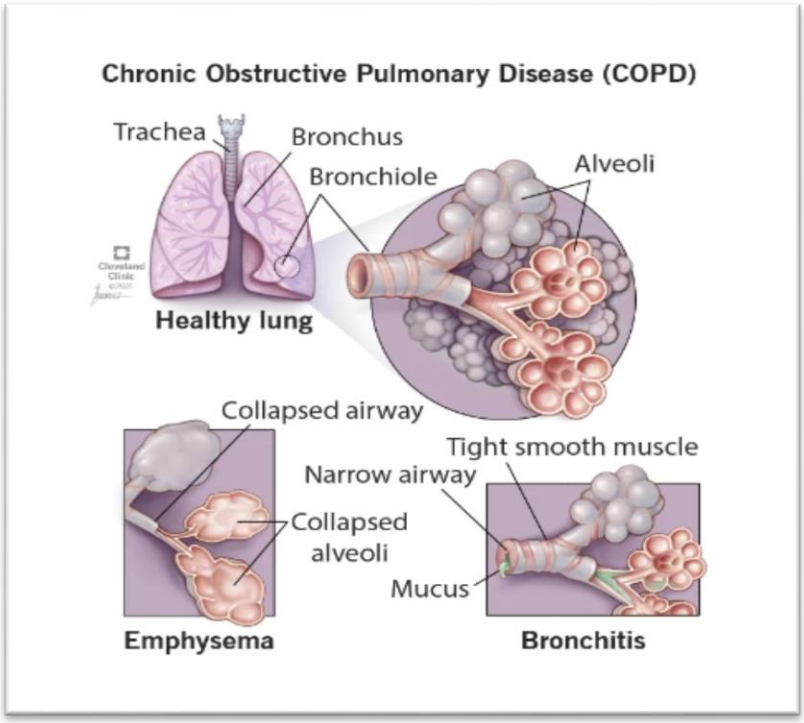


Fig 3: Healthy lungs have open airways versus the collapsed and narrow airways of emphysema and bronchitis, conditions grouped under COPD. (**Source**: https://my.clevelandclinic.org/health/diseases/8709-chronic-obstructive-pulmonary-disease-copd)

2.6 Pathobiology of COPD

COPD is a chronic inflammatory disease. Inhalation of smoke and pollutants leads to chronic airway inflammation by activating the inflammatory cells of the lungs. Due to this, chemotactic signals are released, which recruit more inflammatory cells in the lungs, perpetuating chronic inflammation, which is regarded as the reason for airway obstruction and structural changes. Abnormal inflammation persists in COPD-susceptible patients, and chronic inflammation persists even after smoking cessation(MacNee *et al.*, 2007, Rebe *et al.*, 2007)

A complex array and simultaneous interaction of different inflammation pathways are involved in the development of COPD. Inhalation of tobacco smoke, noxious particles and other harmful gases leads to the activation of inflammatory cells in the lungs that produce chemokines and cytokines that play a vital role in lung parenchyma destruction and chronic inflammation. Epithelial cells are

activated to produce inflammatory mediators such as interleukin-1b(IL-1b), granulocyte monocyte colony-stimulating factor (GM-CSF), tumour necrosis factor α (TNF-α) and interleukin-8 (IL-8). The concentration of activated neutrophils remains high in the sputum and bronchoalveolar lavage of the lungs. Many chemotactic signals have been identified that play a significant role in the further recruitment of neutrophils in COPD, such as IL-8, CXCL1, GRO-a (growth-related oncogene-a) etc. These factors are generally derived from macrophages and epithelial cells, but neutrophils play an essential role in producing IL-8 (Baraldo *et al.,* 2004, Barnes *et al.,* 2003, Traves *et al.,* 2002).

The number of macrophages is marked as 5-10 folds increased in COPD patients' lung parenchyma, sputum and BAL fluid. Macrophages are generally located at the site of emphysema, causing wall destruction. The severity of the disease and the macrophage concentration can be co-related (Finkelstein *et al.,* 1995). Cigarette smoke activates macrophages to produce various inflammatory factors and reactive oxygen species, which are heavily involved in tissue destruction due to apoptosis. Macrophages also produce MMP-2, MMP-9 and MMP-12, proteinases involved in lung tissue remodelling. Macrophages are also involved in the recruitment of monocytes (Punturieri *et al.,* 2000, Russell *et al.,* 2002).

In recent studies, the involvement of NLRP3 inflammasome was found. NLRP3 inflammasome is a multimeric protein complex that is involved in the activation of pro-caspase-1 and the release of IL-1β and IL-18 (Birrell *et al.,* 2011). Smoke inhalation causes an uprise in the body's ROS concentration, which is responsible for activating the inflammasome, recognized through pathogen-associated molecular patterns (PAMPs) (Ishii *et al.,* 2008). PAMPs are recognized by the family of receptors called pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs), etc. TLRs are on the cell surface, while NLRs are inside the cytosol (Opitz *et al.,* 2010, Franchi *et al.,* 2006). PRRs are activated by damage-associated molecular patterns (DAMPs) produced by the cell affected by ROS due to stress generated from smoke inhalation. The activated PRRs activate NLRs by high ATP concentration. Activated NLRs bind with pro-caspase-1 to form inflammasome releasing mature IL-18 and IL-1β, thus further recruiting more inflammatory cells (Mortaz *et al.,* 2009).

High mobility group box 1 (HMGB1) is a chromatin protein that acts as a cytokine when released in the cytoplasm. In COPD patients, the expression of the receptor for advanced glycation end products (RAGE), which binds to HMGB1, was also raised in airway epithelial tissues. The elevated level of HMGB1 complex can even be suggested as a biomarker for COPD. This activated complex activates the PRRs on neutrophils and monocytes, producing further ROS and proteolytic enzymes (MMPs), producing further tissue damage and inflammation (Ferhani *et al.*, 2010, Mitola *et al.*, 2006, Sukkar *et al.*, 2012).

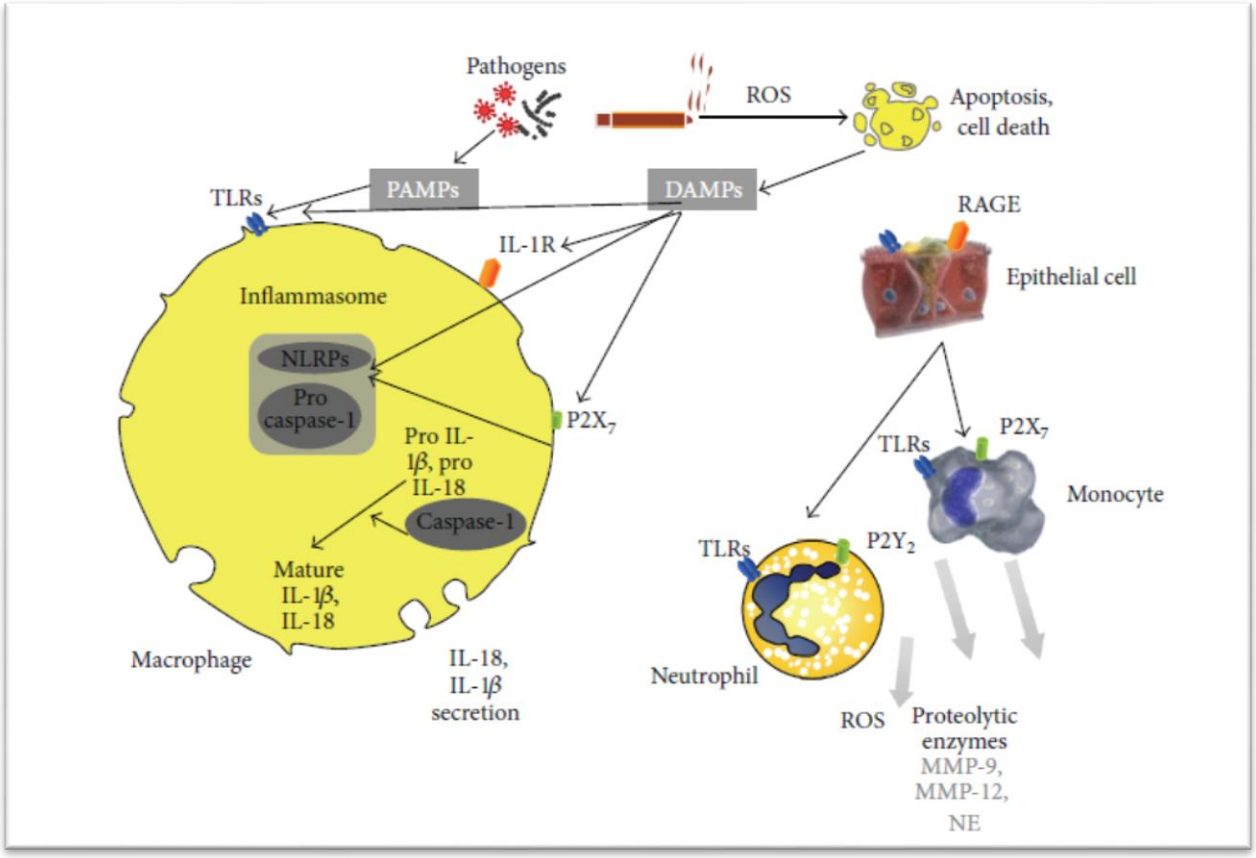


Fig 4: Inflammasome-associated inflammation in COPD.(Rovina *et al.*, 2013)

2.7 Antioxidant enzymes

So, the major conclusion drawn from the pathobiology and progression of COPD is that the root cause of ROS generation and production in the body promotes mutation, apoptosis and inflammation. Thus, many groups of antioxidant enzymes are important in neutralizing excess ROS concentration. Catalase, Superoxide dismutase (SOD), and Glutathione peroxidase (GPx) are the major players in this process. The catalytic properties and substrates are different for each of them, neutralizing different species of ROS. The activation of these enzymes is regulated by nuclear erythroid-2-related factor-2 (Nrf2), which is generally downregulated in COPD patients.(Malhotra *et al.,* 2008)

- Catalase: Catalase is one of the major enzymes that decompose hydrogen peroxide(H2O2), a non-radical form of ROS, into water molecules. It maintains the level of H_2O_2 as a lesser concentration is essential for cell signalling. Humans have typical mono-functional heme-containing catalase. (Nandi *et al.,* 2019)
- Superoxide dismutase family: The family of superoxide dismutase have three isoenzymes Cu-Zn SOD (*SOD1*) present in the cytoplasm, Mn-SOD (*SOD2*) present in the mitochondrial matrix and extracellular SOD (*SOD3*). Among these, cytoplasmic *SOD1* is the most abundant. *SOD3*, like *SOD1*, have metal co-factors such as copper and zinc. They catalyze the dismutation of superoxide radicles to H_2O_2 molecules. *SOD1* accounts for also 85% in the blood of total SOD activity (MacNee 2005).
- Glutathione peroxidase (GPx): GPx is the most abundant thiol-based antioxidant present in cells and plays an essential role in maintaining the redox status of the cells. They are found in abundant quantity in lung fluids and even in extracellular spaces (MacNee 2005).

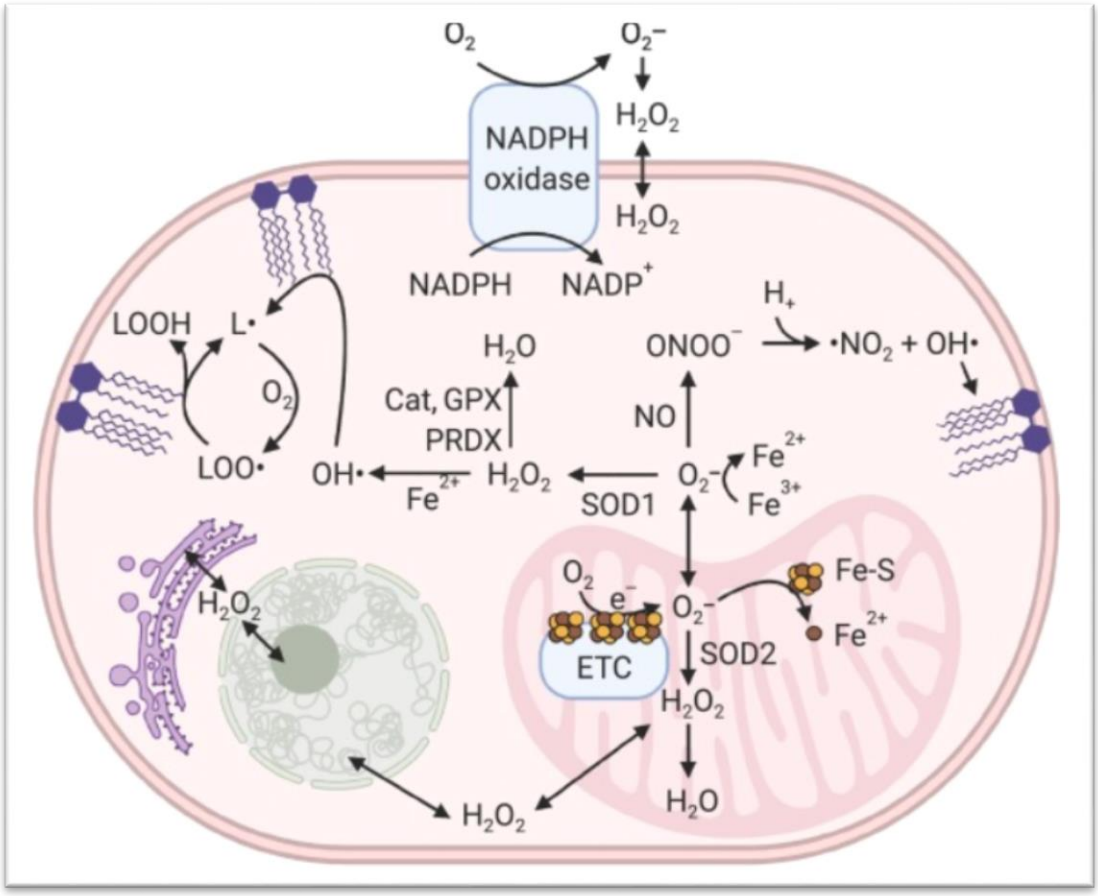


Fig 5: Types of ROS and their neutralization by enzymatic antioxidants. (Harris *et al.*, 2020)

2.8 SOD1 polymorphism in COPD

SODs are the oxidoreductase family of enzymes that catalyze superoxide's dismutation into hydrogen peroxide and oxygen. They play as the first line of defence against ROS along with catalase. In humans, SODs are found in three isoforms – *SOD1*, *SOD2* and *SOD3* (Eskandari *et al.*, 2014).

2.8.1 SOD1 gene

The SOD1 gene is located on chromosome 21q22.11. Cu-Zn SOD, the most abundant SOD, is a soluble cytoplasmic protein. The gene consists of five exons, is interrupted by four introns, and is 9.3 kb based on restriction mapping. The latest genomic assembly showed that the gene contains 9266 bases (Eskandari *et al.*, 2014).

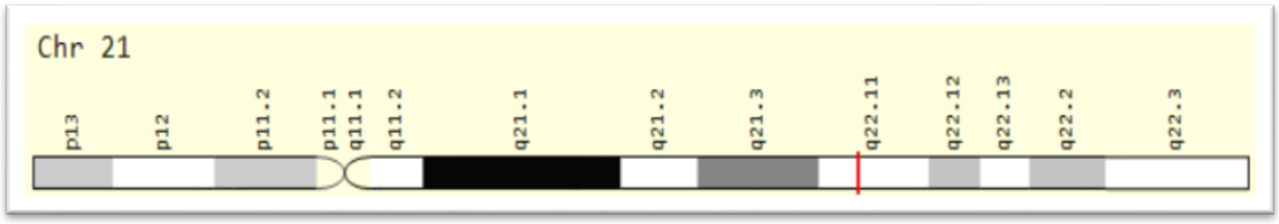


Fig 6: Chromosome 21 showing gene loci of *SOD1*. (Source: https://www.genecards.org/cgi-bin/carddisp.pl?gene=*SOD1*)

2.8.2 SOD1 structure

SOD1 (EC 1.15.1.1) is a 154 amino acid-long protein weighing 15.936 kDa. Two metal co-factors associated with *SOD1* are copper (Cu) and zinc (Zn). It contains only one unique protein chain. The quintenary structure of the protein shows it to exist as a homodimer with non-disulfide linkages. The homodimerization takes place via the di-tryptophan cross-link at Trp-33. The binding of zinc promotes protein dimerization, thus stabilizing the native form. In the absence of intermolecular di-sulfide linkages, the poses a tendency to form fibrillar aggregates. These may pose the tendency to produce cytotoxic effects. Main Transcription factor binding sites in the *SOD1* gene promoter are aMEF-2 C/EBPα, CUTL1, HFH-1, MEF-2A, Myo-D, p53, Pax-4a and RSRFC4 (Sala *et al.*, 2019).

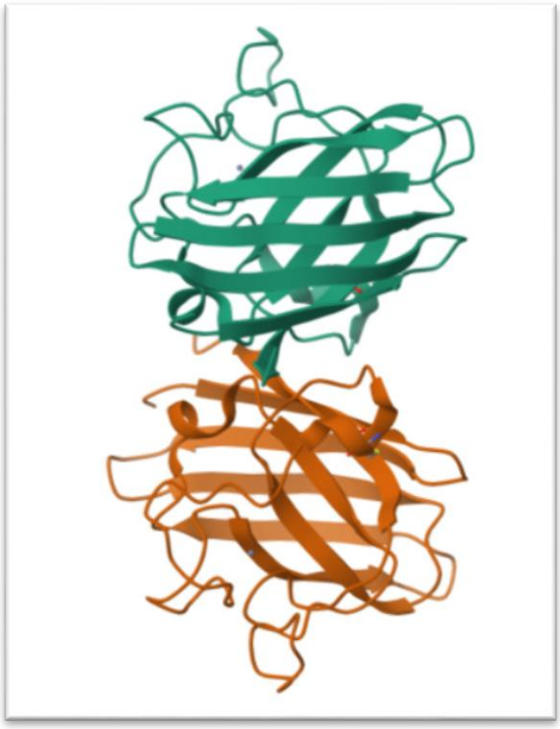


Fig 7: Human *SOD1* structure. (Homodimer) (Sala *et al.*, 2019).

2.8.3 50bp Ins/Del polymorphism of SOD1

The location for this polymorphism is 1684 bp upstream of the ATG start codon. The promoter region is essential as it contains sites for controlling mRNA concentrations and binding sites for transcriptional factors. In-vitro analysis of 50 bp deletion polymorphism showed that the promoter activity was reduced and thus resulting in lower concentrations of mRNA in the cells. This polymorphism is responsible for losing two Sp1 sites(Sarabandi *et al.,* 2022). Sp1 is a zinc finger transcription factor which binds to G.C.-rich motifs of the promoter region, promoting cellular growth and differentiation, immune response, apoptosis, response to DNA damage and chromatin remodelling. Loss of two Sp1 sites results in lower levels of transcription of *SOD1* genes, thus resulting in lower *SOD1* activity in cells. Deficiency of *SOD1* results in the rise of superoxide. SOD1 50 bp Ins/Del polymorphism has been shown to contribute to the pathogenesis of CVDs and bladder cancer risk in the Iranian population(Eskandari *et al.,* 2014, Darvishi *et al.,* 2019).

Fig 8: PCR amplified product for checking 50 bp Ins/Del polymorphism in *SOD1*. Green highlighted segments are the binding sites for forward and reverse primers. The deleted region started from nucleotide number 113 of the PCR product. (Darvishi *et al.*, 2019)

2.9 Catalase polymorphism in COPD

Catalase (EC 1.11.1.6) is one of the most important antioxidant enzymes, the body's defence against oxidative stress. Catalase is a heme-containing enzyme in the peroxisomes of almost all aerobic organisms. H_2O_2, a toxic ROS species, is neutralized by catalase to produce water and oxygen (Wang *et al.*, 2016).

2.9.1 Catalase gene

The catalase gene is located on chromosome 11p13.31. The gene consists of thirteen exons, is interrupted by twelve introns, and is 32.4kb based on restriction mapping. The latest genomic assembly showed that the gene contains 33127 bases. The main transcription factor binding sites in catalase gene promoter are FOXO1, FOXO1a, GR, GR-α, GR-β, NF-κβ and NF-κβ1 (Wang *et al.*, 2016).

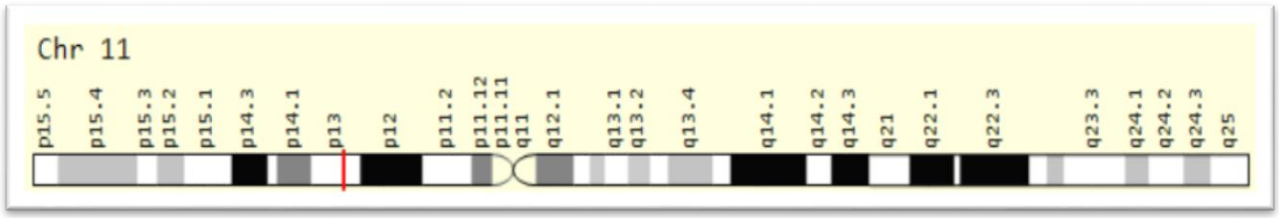


Fig 9: Chromosome 21 showing gene loci of *SOD1*. (Source: https://www.genecards.org/cgi-bin/carddisp.pl?gene=CAT#genomic_location)

2.9.2 Catalase structure

Catalase is a 527 amino acid long protein weighing 59.75 kDa. Two co-factors that are associated with catalase are heme and $NADP^+$. The quintenary structure of the protein shows it to exist as a homo-tetramer showing interaction with PEX5, leading to its translocation into peroxisomes (Putnam *et al.*, 2000).

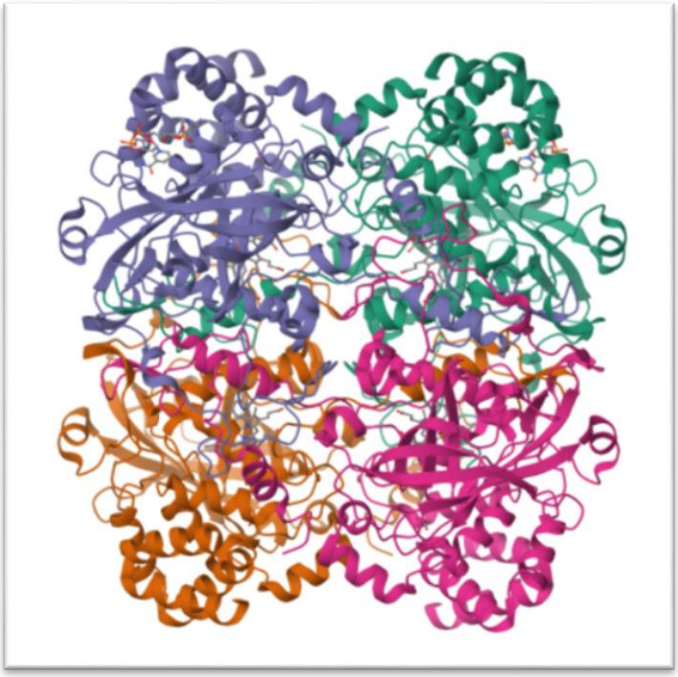


Fig 10: Human catalase structure. (Homo-tetramer) (Putnam *et al.*, 2000).

2.9.3 262 C>T polymorphism of catalase

This is a common polymorphism present in the promoter region of the catalase gene. Substitution occurs in the 5' untranslated region, at -262 position C to T. This polymorphism affects the transcriptional factor binding in the promoter region and splicing regulation (Wang *et al.*, 2016). The T allele has shown lower enzyme activity than the C allele. Due to lower levels of an enzyme in the cells, the ROS concentration rises, producing toxic effects hampering tissue and cellular health. As more significant concentrations of ROS remain un-neutralized, the prevalence of chronic inflammation worsens, thus, amplifying the effect of the disease. This polymorphism has been shown to contribute to cancer pathogenesis (Eras et al ., 2019 Taniguchi *et al.*, 2014).

2.10 Diagnosis and severity standards of COPD

Diagnosis of COPD should be done by first monitoring and checking for the symptoms such as breathlessness, chronic cough and mucus production with mandatory history of exposure to the risk factors. The mandatory factor in establishing the diagnosis is the presence of post-bronchodilator FEV1/FVC < 0.7 in forced Spirometry.(Buist *et al.,* 2007)

2.10.1 Spirometry

Gold Initiative for Lungs Disease (GOLD) defines definite COPD based on forced spirometry values. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have endorsed the GOLD definition, which uses a fixed ratio of the postbronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) of less than 0.70 to define obstruction (van Dijk *et al.,* 2015). Forced expiratory volume is the air a person can forcefully exhale in the first seconds of expiration after optimal inspiration. FVC is the maximum amount of air that the lungs can expel in "t" seconds that it is empty. Generally, this time varies from 3-15 seconds, depending on the disease severity. This criterion of diagnosis using FEV1 and FVC values is independent of reference values because the variables being measured are from the same individual, and the treatment standards were drawn from the data produced from all the clinical trials performed in this sector (Güder *et al.,* 2012). One drawback of using a fixed FEV1/FVC ratio (< 0.7) to conclude obstruction can result in under-diagnosis in young adults and over-diagnosis in aged people, especially in those individuals with mild, compared to using a cut-off based on the lower limit of normal (LLN) values for FEV1/FVC. The values of LLN are based on the normal distribution and selectively organize the bottom 5% of the healthy population as an obstructed condition. Spirometry is the GOLD standard for diagnosing, monitoring and staging the disease due to its accurate, noninvasive, reproducible, cheap and readily available test (Bhatt *et al.,* 2019). It is recommended that the assessment of the presence or absence of obstruction for FEV1/FVC values between 0.60 to 0.80 should be confirmed by repeated Spirometry on a different occasion because of the fact or a chance of the ratio change due to biological variation when measured at a different interval. Spirometry should be done pre- and post-bronchodilation for each individual after being given a short-acting inhaled bronchodilator to compare and rule out the possibilities of reversibility (to rule out the possibility of asthma) (Rebe *et al.,* 2007).

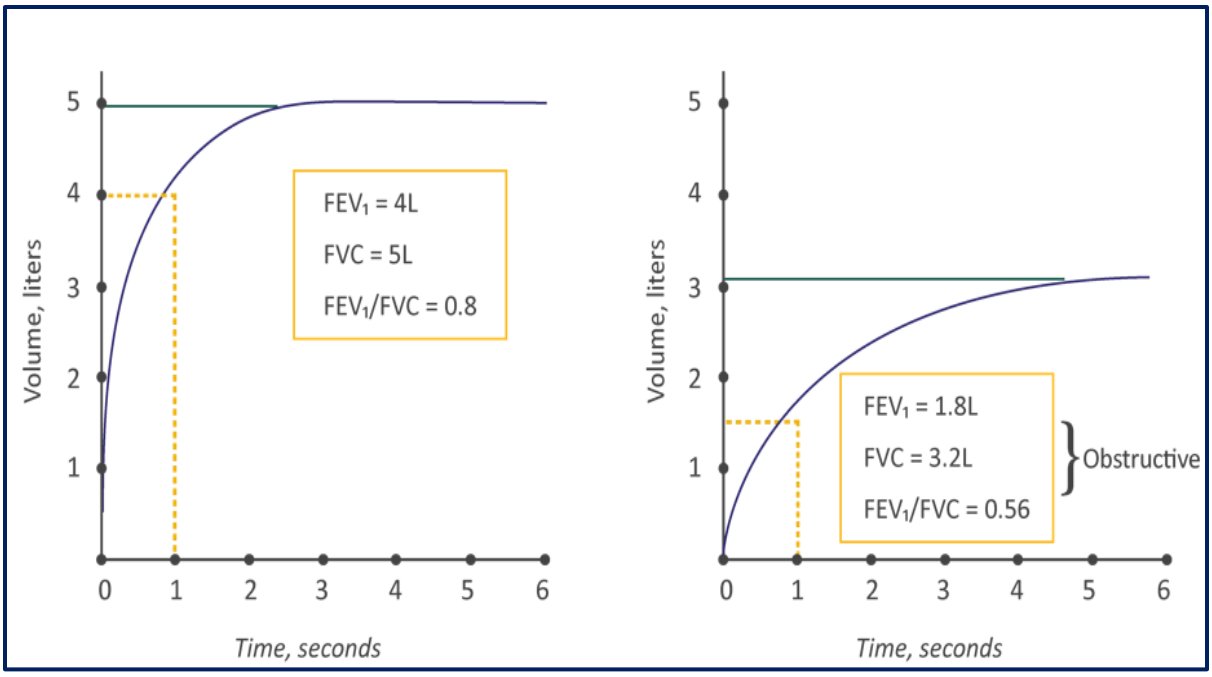


Fig 11: Graphs depicting spirometry changes due to obstruction in average (left) vs diseased individual (right) showing lowered FEV1 and FVC values (Rebe *et al.*, 2007).

2.10.2 Severity Standards of COPD

The disease's severity is judged on a lower % FEV1 value based on specific cut points and standards. The criterion to evaluate the severity is first based on the presence of FEV1/FVC ratio <0.7.

- Gold Severity: It is based on assigning severity grades based on the %FEV1 value. The lower the value higher, the severity of the disease (Rebe *et al.*, 2007).

GOLD stage	Severity	Spirometry
I	Mild	FEV1/FVC <0.7 and FEV1 ≥80% predicted
II	Moderate	FEV1/FVC <0.7 and FEV1 ≥50% but <80% predicted
III	Severe	FEV1/FVC <0.7 and FEV1 ≥30% but <50% predicted
IV	Very severe	FEV1/FVC <0.7 and FEV1<30%

Fig 12: Gold severity grading for airflow obstruction in COPD (Rebe *et al.*, 2007).

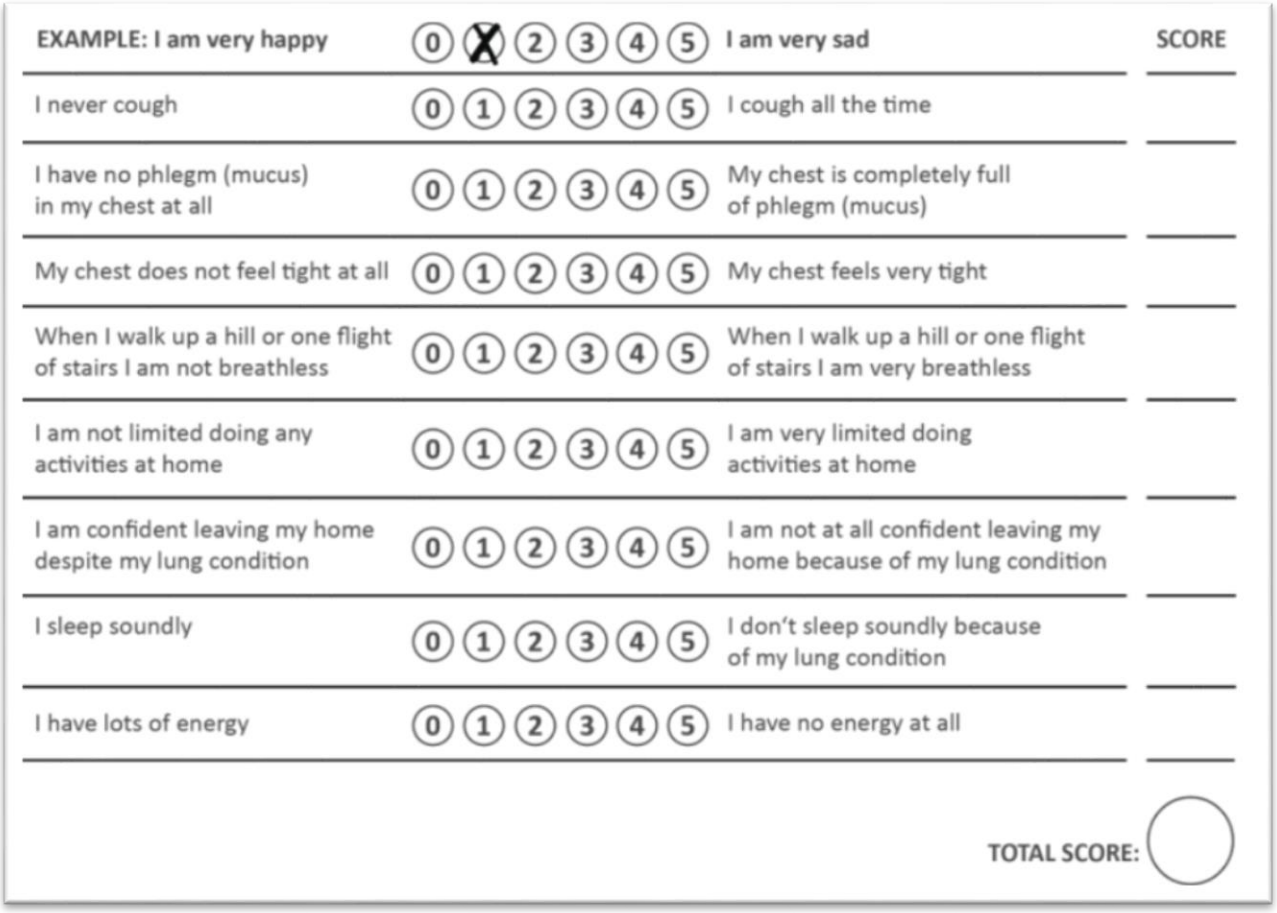
- mMRC scale: mMRC was the first scale questionnaire developed to measure the most common symptom of the disease: breathlessness (Rebe *et al.*, 2007).

mMRC Grade 0.	I only get breathless with strenuous exercise.
mMRC Grade 1.	I get short of breath when hurrying on the level or walking up a slight hill.
mMRC Grade 2.	I walk slower than people of the same age on the level because of breathlessness, or I have to stop for breath when walking on my own pace on the level.
mMRC Grade 3.	I stop for breath after walking about 100 meters or after a few minutes on the level.
mMRC Grade 4.	I am too breathless to leave the house or I am breathless when dressing or undressing.

Fig 13: Modified MRC breathlessness scale (Rebe *et al.*, 2007).

Due to recognizing more acute symptoms other than breathlessness, multidimensional questionnaires are now recommended.

- CAT score: COPD Assessment Test (CAT) is an eight-listed specification questionnaire that evaluates the patient's health status. The score ranges from 0 to 40, where the higher the severity of the disease, the higher will the score assigned (Karloh *et al.*, 2016).



EXAMPLE: I am very happy	0	X	2	3	4	5	I am very sad	SCORE
I never cough	0	1	2	3	4	5	I cough all the time	
I have no phlegm (mucus) in my chest at all	0	1	2	3	4	5	My chest is completely full of phlegm (mucus)	
My chest does not feel tight at all	0	1	2	3	4	5	My chest feels very tight	
When I walk up a hill or one flight of stairs I am not breathless	0	1	2	3	4	5	When I walk up a hill or one flight of stairs I am very breathless	
I am not limited doing any activities at home	0	1	2	3	4	5	I am very limited doing activities at home	
I am confident leaving my home despite my lung condition	0	1	2	3	4	5	I am not at all confident leaving my home because of my lung condition	
I sleep soundly	0	1	2	3	4	5	I don't sleep soundly because of my lung condition	
I have lots of energy	0	1	2	3	4	5	I have no energy at all	

TOTAL SCORE:

Fig 14: CAT assessment questionnaire (Rebe *et al.*, 2007).

- Gold Group (ABE): In 2011, GOLD proposed to change the grading of severity of the disease simply from a spirometric grading system to a combinatorial method based on the level of symptoms (mMRC and CAT), the severity of airflow obstruction (Gold severity FEV) and the frequency of previous worsening symptoms. In 2023, the earlier ABCD grading of severity was changed to ABE grading by GOLD. A and B groups in the new grading remained the same, while C and D were combined to form E. Here A group shows minor severity while group E showed the highest severe conditions (Rebe *et al.*, 2007).

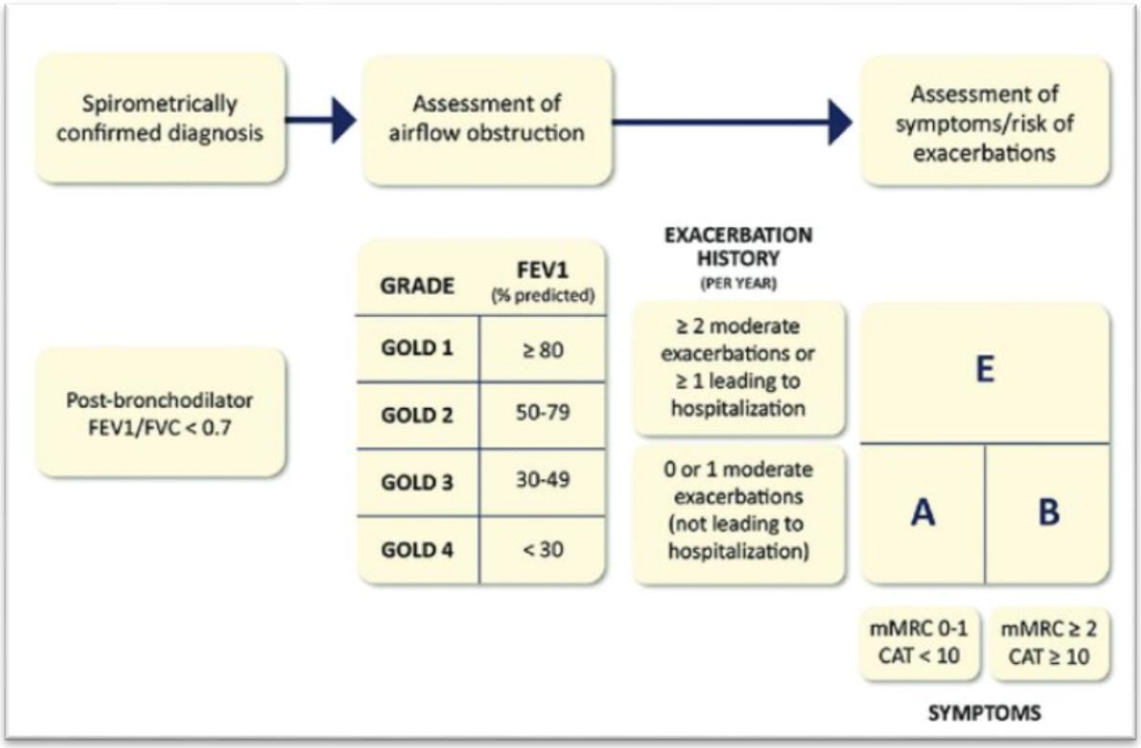


Fig 15: GOLD ABE assessment tool (Rebe *et al.*, 2007).

2.11 Comorbidities

COPD often coexists with other diseases, impacting or worsening the health condition. Few may arise with COPD, while others may be independently related. There might also be the complementing of the risk factor of both diseases, which can exist as a compounding effect of symptoms of one disease over another. Comorbidities are irrespective of the severity of COPD and can hamper differential diagnosis. Specific combinatorial effects of these diseases have resulted in higher morbidity and mortality rates in COPD patients. Some common examples are lung cancer, cardiovascular diseases, ischaemic heart disease, arrhythmias, hypertension, metabolic syndromes, osteoporosis, anaemia, anxiety and depression, etc (Divo *et al.*, 2012, Fabbri *et al.*, 2008).

- Lung cancer: COPD is commonly comorbid with lung carcinoma due to the oxidative stress generated and the DNA damage associated with it. Prolonged inflammation, the suppression of DNA repair due to high ROS generation, and epigenetic changes in DNA methylation are potential contributors to the development of lung cancer in COPD patients.

Both COPD and lung cancer have the exact root cause of tobacco smoking. The common risk factors involved in the development of lung cancer in COPD patients include:

  - Smoking history of more than 30 packs a year
  - Age above 55
  - Presence of emphysema
  - Spirometry FEV/FVC value <0.7
  - Family history of having lung cancer (Caramori *et al.,* 2011).

- Cardiovascular diseases: It is evident that cardiovascular diseases (CVDs) cause the highest number of deaths per year. COPD patients are highly susceptible to CVDs. It has also been reported that mortality due to comorbid CVDs in COPD patients is higher than respiratory failures. Types CVDs prevailing in COPD patients are:
  - Hypertension is the most commonly found comorbidity and may have implications for prognosis.
  - Heart failure: Heart failure ranges from 20% - 70% in COPD patients.
  - Arrhythmias: Atrial fibrosis is often observed as a cause of lower FEV1, making it a common comorbidity of COPD
  - Ischemic heart disease: Depending on the risk factor, ischemic heart disease can be considered for almost all COPD patients (Dransfield et., al 2011).

2.12 Gaps in the literature

Very few studies have been conducted on the involvement of SNPs for *SOD1* and *catalase* in the lungs and its association and involvement in pathogenesis in COPD patients. The clinical outcomes involving the SNPs of these genes are yet to be discovered correctly in the disease prognosis. In India, no studies have been conducted for the prognosis of the disease involving these SNPs. As the Indian population is one of the highest suffers from COPD, it becomes necessary to study the clinal outcomes in the patients. The deleterious effect of these mutations can be identified as biomarkers and helpful in early diagnosis and treatment of patients preventing the disease prognosis. Early diagnosis and knowledge of expected clinical outcomes can provide better ideas of disease severity, thus, better developments of treatment strategies.

CHAPTER 3

OBJECTIVES:

1. To evaluate the role of genetic variation in the Superoxide dismutase-1 (*SOD1*) gene and its risk towards COPD
2. To check the role of the genetic polymorphism in the catalase (*CAT*) gene and its risk towards modulating COPD

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION

This study accounts for 200 Chronic Obstructive Pulmonary Disease (COPD) patients and 200 controls (total of 400) recruited from the Department of Pulmonary Medicine, Government Medical College (GMC), Patiala, India. This study has been reviewed and approved by the institutional ethics committees of Government Medical College (GMCP), Patiala and Thapar Institute of Engineering and Technology. For all subjects diagnosed with COPD, informed written consent was obtained from them or their representatives. Each patient completed a detailed questionnaire containing demographic and smoking characteristics such as tobacco habits like smoking beedi/cigarette etc. The subjects having a regular smoking habit were classified as smokers. The clinic-pathological details such as FEV value, CAT score, MMRC, Gold severity, Gold group, smoking period, symptoms, smoking gap and biomass exposure were obtained from the hospital records. Gender and other parameters were also taken into consideration. Approximately 3-5mL of venous blood was collected from each participant in an EDTA-coated vacutainer and stored at 4°C until DNA extraction.

4.2 DNA ISOLATION

Genomic DNA was isolated from blood samples of COPD patients using standard Proteinase-K digestion, phenol-chloroform extraction and isopropanol precipitation (Bartlett and White, 2003).

Requirements:

- Washing buffer
- Lysis buffer
- Phenol: Chloroform: Isoamyl alcohol (25:24:1)
- Chloroform: Isoamyl alcohol (24:1)
- Isopropanol
- Ethanol
- T.E. buffer

Buffer preparation:

Washing buffer, lysis buffer and T.E. buffer were prepared as shown in the table:

STOCK CONCENTRATION	WORKING CONCENTRATION
1M Sucrose	320mM Sucrose
100% Triton-X-100	1% Triton-X-100
100mM Magnesium Chloride	5mM Magnesium Chloride
100mM Tris-HCl (pH 8.0)	10mM Tris-HCl (pH 8.0)

Table 4.2.1 Preparation of Washing buffer

STOCK CONCENTRATION	WORKING CONCENTRATION
1M Tris-HCl (pH 8.0)	400mM Tris-HCl (pH 8.0)
10% SDS	1% SDS
0.5M EDTA	60mM EDTA
2M NaCl	150mM NaCl
10mg/mL Proteinase- K	100µg/mL Proteinase- K

Table 4.2.2 Preparation of Lysis Buffer

The procedure of DNA isolation:

- Blood and chilled washing buffer were mixed in a 1:1 ratio and mixed thoroughly. The tubes were incubated in ice for 1 minute and centrifuged at 5000 RCF for 5 minutes at 4°C.
- The supernatant was carefully discarded, and chilled washing was again added in a 1:1 ratio and vortexed. The tubes were then incubated in ice for a minute and centrifuged at 5000 RCF for 5 minutes at 4°C. (Repeated this step; in total thrice).
- The pellet was dissolved in lysis buffer in a 1:1 ratio and vortexed. The tubes were incubated at 46°C overnight.
- An equal volume of Phenol: Chloroform: Isoamyl alcohol (PCI) (25:24:1) was added and mixed gently. The tubes were centrifuged at 8000 RCF for 10 mins at 4°C.

- The clear aqueous layer supernatant was separated, and equal PCI volumes were added and mixed slowly. The tubes were centrifuged at 8000 RCF for 10 mins at 4°C.
- The clear aqueous layer supernatant was separated, and equal volumes of Chloroform: Isoamyl alcohol (24:1) was again added and mixed slowly. The tubes were centrifuged at 6500 RCF for 10 mins at 4°C.
- To the transparent aqueous layer, equal volumes of chilled isopropanol were added and mixed gently. Freeze the tubes at -20°C for 1-2 hours.
- They were centrifuged at 12000 RCF for 10 mins at 4C. The supernatant was discarded, and the pellet was washed with 70% ethanol twice at 12000 RCF for 5 mins at 4°C.
- Ethanol was discarded, and the pellet was allowed to air dry.
- The pellet was then dissolved in 70-140 µL of Tris- EDTA buffer, depending on the size of the pellet.

4.3 QUANTITATIVE ESTIMATION OF DNA

The quantitative DNA estimation was done using UV-Spectrophotometer, and the absorbance was recorded at two wavelengths, A260 nm and A280 nm. The ratio of absorbance at 260nm and 280 nm is used to assess the purity of DNA. DNA concentration of the solution was determined by using the formula:

DNA Concentration (µg/ml) = O.D at 260nm x 50 x Dilution factor

Where 50µg/ml of DNA is equal to 1 O.D

Purity of DNA = O.D at 260nm/ O.D at 280nm

NOTE: If the ratio of A260/ A280 equals 1.8, then it is said to have pure DNA without contamination. The ratio is more significant than 1.8, close to 2, and it is said to be contaminated with RNA. And if the ratio is less than 1.8, it is said to be contaminated with protein, phenol or other contaminants.

Procedure

- 4 µL of each isolated DNA sample were made up to 1000 µL using Tris-EDTA buffer.
- Absorbance was taken on a spectrophotometer at A260/ A280 against Tris- EDTA buffer as blank.
- Quantitative and purity analyses of each sample were done automatically by the instrument.

4.4 QUALITAIVE ANALYSIS OF DNA

Requirements:

- Gel casting tray
- Gel comb
- Horizontal gel electrophoresis apparatus
- Electrophoresis running buffer (TAE or TBE buffer)
- Electrophoresis grade agarose
- Ethidium bromide solution
- 6X loading dye
- DNA molecular weight marker
- D.C. power supply

Preparation of 5X TBE

Name of the component	Amount
Tris base	54g
Boric Acid	27.5g
EDTA (0.5M)	20 ml

The required final volume was made up of distilled water.

Preparation of 6X Loading Dye

Name of the component	100ml	50 ml
30% Sucrose	30g	15g
0.1% Bromophenol Blue	0.1g	0.05g
20mM EDTA	4 ml	2ml

Preparation of Agarose gel for electrophoresis

- We have prepared an adequate volume of electrophoresis running buffer by diluting 5X TBE to 0.5X with distilled water.
- The desired amount of electrophoresis-grade agarose was weighed and mixed with the appropriate amount of 0.5X TBE. For example: for the resolution of genomic DNA, 0.8% gel was prepared (mixed 0.8g of agarose to 100mL of 0.5X TBE buffer).
- The mixed agarose suspension was then heated to a boil to form a solution. The solution was then allowed to cool to approximately 55°C.
- Before pouring the gel, Ethidium bromide solution was added to a final concentration of 0.3µg/mL to facilitate visualization of DNA when viewed under UV-transilluminator.
- The melted agarose solution was poured onto an approximately 0.5 to 1.0 cm gel casting tray. A comb was inserted before pouring the agarose solution, ensuring that there should be no bubble trapped underneath the comb or the surface of the gel to be solidified and set.

Loading and running of gel

- The comb was carefully removed from the solidified gel without disturbing the formed sound structure.
- The casting tray and gel were placed in the electrophoretic tank, and a sufficient amount of the electrophoretic running buffer was added to submerge the gel completely. Air pockets trapped in the wells were removed using a fresh tip.
- For loading, 2µL of DNA sample, 1µL of dye and 3µL of water were mixed and loaded sequentially in individual wells.

- The electrodes were then connected to the power pack and allowed to run at 70V and 25mA till the dye front reached two-thirds length of the gel.
- The unit was then switched off, and the electrophoretic buffer was discarded.
- DNA was then visualized under a UV-transilluminator and photographed using Gel documentation.

4.5 GENOTYPING OF POLYMORPHIC SITES IN *SOD1* AND *CATALASE* GENES.

4.5.1 GENOTYPING OF *SOD1* POLYMORPHISM BY PCR

Genotyping analysis of insertion/deletion polymorphism of *SOD1* was performed using the PCR technique. Standard PCR amplification of the polymorphic sites using primers and DNA polymerase enzyme was done. The amplification was carried out in a 15µL reaction mixture. For determining the annealing temperature of the primers, first, the neb Tm calculator was used, and then the calculated Tm was checked using the gradient PCR technique. After the amplification, the samples were checked using 2.0% agarose gel, using ethidium bromide staining for DNA band visualization under UV-transilluminator and gel documentation.

GENE	POLYMORPHISM	POLYMORPHISM ID#	PRIMERS	PCR PRODUCT (bp)	FRAGMENTS IDENTIFYING GENOTYPES (bp)
SOD1	50bp Ins/Del in *SOD1* promoter	757439	5'- AAT TCC TTA CCC CTG TTC TA-3' Forward Primer 5'- GGC AGA TTT CAG TTC ATT GT-3' Reverse primer	297	Ins/Ins- 297 Ins/Del- 297 & 247 Del/Del- 247

TABLE 4.5.1.1 List of primers and amplified products for *SOD1* genetic variants.

Requirements:

- Water
- BSA
- 10X PCR buffer
- Forward primer
- Reverse Primer
- dNTPs
- Taq DNA polymerase

COMPONENTS	STOCK CONCENTRATION	WORKING CONCENTRATION	VOLUME USED FOR 1 REACTION
Water			10.0 µL
BSA	100X	10X	1.5 µL
Buffer	10X	1X	1.5 µL
Forward primer	10µM	0.5 µM	0.75 µL
Reverse primer	10 µM	0.5 µM	0.75 µL
dNTPs	10 µM	0.2 µM	0.3 µL
Taq polymerase	5U	1U	0.2 µL
DNA template			0.50 µL

TABLE 4.5.1.2 Concentration of requirements of Polymerase Chain Reaction.

S.No.	Steps	Temperature	Time
1.	Initial denaturation	95°C	5 mins
2.	Denaturation	94°C	0.10 mins
3.	Annealing	50°C	0.45 mins
4.	Polymerization	72°C	0.30 mins
5.	Final Extension	72°C	5 mins

Steps 2, 3 & 4 are repeated for 30 cycles.

TABLE 4.5.1.3 Temperature and time specifications for steps of Polymerase Chain Reaction.

Procedure:

- All the required reagents were thawed.
- The master mix was prepared by diluting all the reagents in the required concentration for an adequate number of reactions required.
- 15 µL of the master mix was pipetted into each PCR tube, and then a DNA template was added.
- The content of the PCR tubes was gently tapped and quickly spun.
- The tubes were then transferred to the thermocycler.
- After the PCR cycle was complete, each sample was subjected to 2% agarose gel electrophoresis and the results were viewed under a U.V. transilluminator.

4.5.2. GENOTYPING OF *CATALASE* POLYMORPHISM BY PCR-RFLP

Genotyping analysis of 262 C>T polymorphism was performed using the PCR-RFLP technique. Standard PCR amplification of the polymorphic sites using primers and DNA polymerase enzyme was done. The amplification was carried out in a 15µL reaction mixture. For determining the annealing temperature of the primers, first, the neb Tm calculator was used, and then the calculated Tm was checked using the gradient PCR technique. On the confirmation of PCR, the amplified products were subjected to restriction cutting using the enzyme *SmaI*. The results were recorded in agarose gel of 2% under a U.V. transilluminator.

GENE	POLYMORPHISM	SNP ID	PRIMERS	PCR PRODUCT SIZE (bp)	RESTRICTION ENZYMES	FRAGMENTS IDENTIFYING GENETYPES (bp)
Catalase (CAT)	262 C>T	1001179	5'- AGA GCC TCG CCC CGC CGG ACC G-3' Forward Primer	185	*SmaI*	CC- 155,30 CT- 185,155,30 TT-185

			5'- TAA GAG CTG AGA AAG CAT AGC T-3' Reverse primer			

TABLE 4.5.2.1 List of primers and restriction enzymes for Catalase genetic variants.

Requirements:

For PCR:

- Water
- $MgCl_2$
- BSA
- 10X PCR buffer
- Forward primer
- Reverse Primer
- dNTPs
- Taq DNA polymerase

For restriction cutting:

- Water
- RE buffer
- SmaI enzyme

COMPONENTS	STOCK CONCENTRATION	WORKING CONCENTRATION	VOLUME USED FOR 1 REACTION
Water			9.55 µL
$MgCl_2$	50mM	1.5mM	0.45 µL
BSA	100X	10X	1.5 µL
Buffer	10X	1X	1.5 µL

Forward primer	10µM	0.5 µM	0.75 µL
Reverse primer	10 µM	0.5 µM	0.75 µL
dNTPs	10 µM	0.2 µM	0.3 µL
Taq polymerase	5U	1U	0.2 µL
DNA template			0.77 µL

TABLE 4.5.2.2 Concentration of requirements of Polymerase Chain Reaction.

S.No.	Steps	Temperature	Time
1.	Initial denaturation	95°C	5 mins
2.	Denaturation	94°C	0.10 mins
3.	Annealing	50°C	0.45 mins
4.	Polymerization	72°C	0.30 mins
5.	Final Extension	72°C	5 mins

TABLE 4.5.2.3 Temperature and time specifications for steps of Polymerase Chain Reaction.

Steps 2, 3 & 4 are repeated for 30 cycles.

COMPONENTS	STOCK CONCENTRATION	WORKING CONCENTRATION	VOLUME USED FOR 1 REACTION
Buffer	10X	1X	2 µL
***SmaI* enzyme**	10U/ µL	1.5U/ µL	0.15 µL
PCR product			10 µL
H_2O			7.85 µL
Total			20 µL

TABLE 4.5.2.4 Reaction mixture for restriction digestion.

Procedure:

- All the required reagents were thawed.
- The master mix was prepared by diluting all the reagents in the required concentration for an adequate number of reactions required.
- 15 µL of the master mix was pipetted into each PCR tube, and then a DNA template was added.
- The content of the PCR tubes was gently tapped and quickly spun.
- The tubes were then transferred to the thermocycler.
- After the PCR cycle was complete, each sample was subjected to 2% agarose gel electrophoresis and the results were viewed under a U.V. transilluminator.
- 2 µL of PCR amplified product was pipetted from each tube and stored uncut.
- The reaction master mix for restriction cutting was prepared by mixing an adequate volume of requirements for the required number of reactions.
- 10 µL of the mix was added to each tube and incubated at 25C overnight (not less than 6hrs.)
- The incubated products were subjected to 2% agarose gel electrophoresis and visualized under a U.V. transilluminator.

4.6 STATISTICAL ANALYSIS

The $\chi 2$ test for categorical data was used to assess differences in the distribution of demographic characteristics between cases and controls. In cases and controls, the Hardy–Weinberg equilibrium theory ($p^2+2pq+q^2=1$; where p is the frequency of the wild-type gene and q is the number of variant alleles) was used to calculate the genotype frequencies of *SOD1 and catalase* genes polymorphism using the χ^2 test. To evaluate the risk of COPD with *SOD1 polymorphisms* and *catalase* polymorphism, logistic regression analysis with adjustment for potential parameters (age, gender and smoking) was used to determine the adjusted odds ratios

(O.R.s) and 95% confidence intervals (C.I.s). All p-values were two-sided, and p-values < 0.05 were statistically significant. Medical version 9.3.6.0 (Medcalc Software, Ostend, Belgium) was used for the statistical study.

CHAPTER 5

RESULTS

5.1 Genomic DNA isolation

After the DNA was isolated from peripheral blood, the samples were subjected to electrophoresis in 0.8% agarose gel containing Ethidium bromide and prepared 0.5X TBE buffer. After the completion of the run, the gel was visualized under a UV transilluminator and gel documentation.

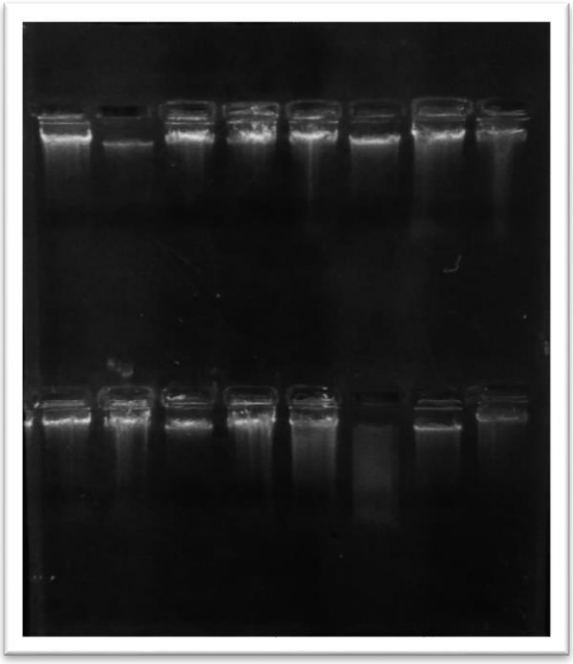


Fig 16: Pictorial representation of 0.8% gel after DNA isolation.

5.2 PCR amplification and polymorphic variants of SOD1

The gene was amplified Using the temperature parameters mentioned in Table 4.5.1.3 and the appropriate forward and reverse primer set. The amplicon was subjected to 2.0% agarose gel electrophoresis with EtBr. Bands of 297 and 247 bps were viewed against UV transilluminator and gel documentation with the help of a suitable DNA ladder.

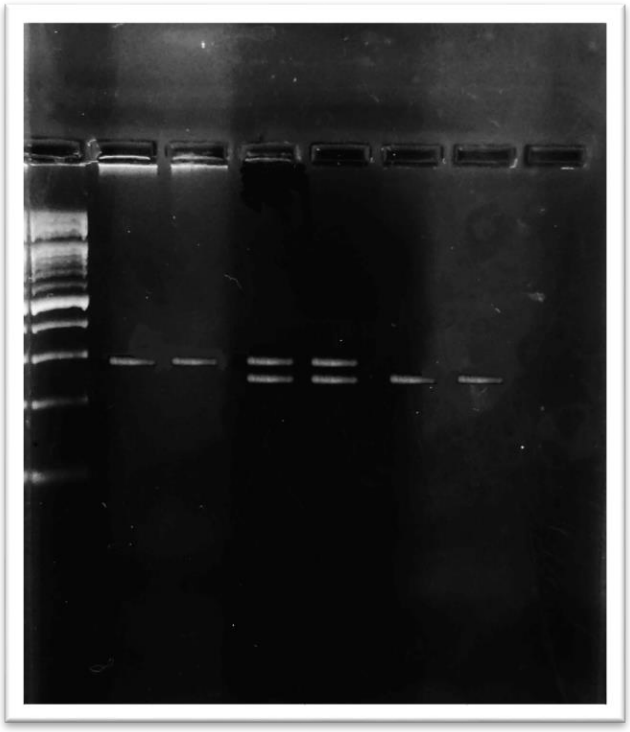


Fig 17: Pictorial representation of PCR amplification product of SOD1 gene on 2% agarose gel.

Well 1 – DNA ladder

Well 2 and 3 – Wild phenotype 297 bps

Well 4 and 5 – Hetero phenotype 297 and 247 bps

Well, 6 and 7 – Mutant phenotype 247 bps

5.3 PCR-RFLP of catalase polymorphism

5.3.1 PCR amplification

The gene was amplified Using the temperature parameters mentioned in Table 4.5.2.3 and the appropriate forward and reverse primer. The amplicon was subjected to 2.0% agarose gel electrophoresis with EtBr. A band of length 185 bp was viewed against a UV transilluminator and gel documentation with the help of a suitable DNA ladder.

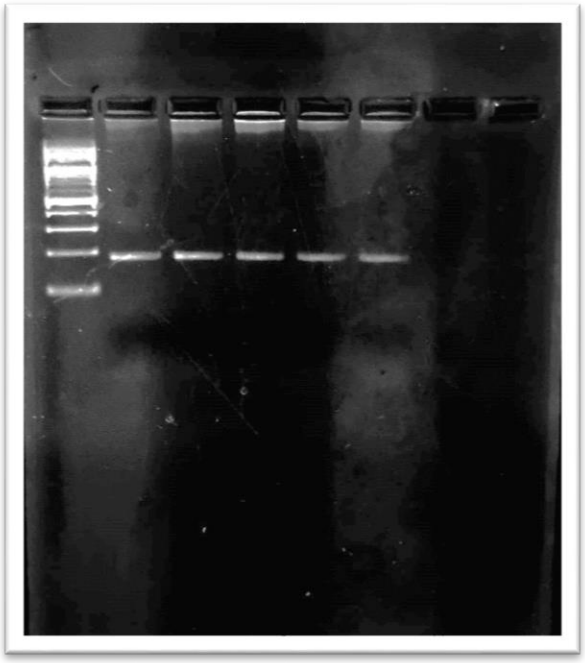


Fig 18: Pictorial representation showing PCR amplification product (bands of 185 bps) catalase gene on 2% agarose gel.

5.3.2 Restriction fragment length polymorphism

The PCR product of the catalase gene upon restriction digestion with Sm1 produced 155 and 30 bps DNA fragments for wild genotypes, 185, 155 and 30 bps for heterozygous genotypes and only 185 bp in the case of mutant genotypes when subjected to 2.0% agarose gel electrophoresis with EtBr. Bands were viewed against UV transilluminator and gel documentation with the help of a suitable DNA ladder.

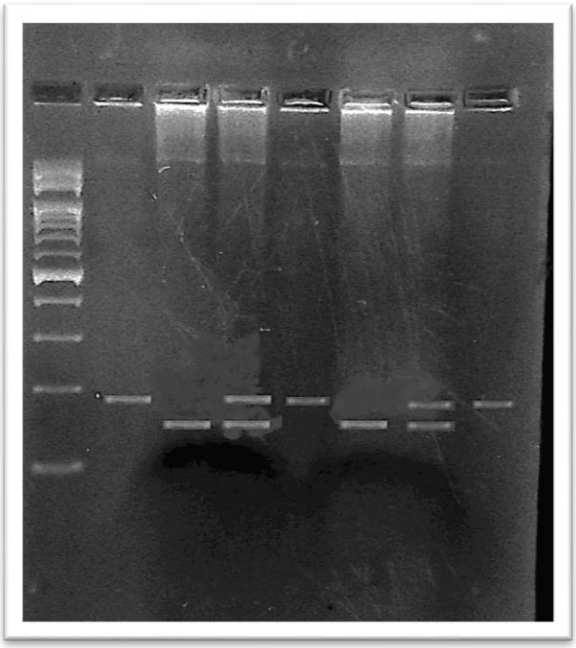


Fig 19: Pictorial representation of PCR amplification product after restriction cutting with SmaI of catalase gene on 2% agarose gel.

Well 1 – DNA ladder

Well 2 – Uncut DNA

Well 3 and 6 – Wild phenotype 155 bps

Well 4 and 7 – Hetero phenotype 185 and 155 bps

Well 5 and 8 – Mutant phenotype 185 bps

5.4 Demographical characteristics of COPD patients.

Parameters	Cases, (n%) N=200	Controls, (n%) N=200	p-value
Age (Years)			**0.2055**
Mean ± SD	58.54 ± 11.425	57.31 ± 7.59	
Range	(21-80)	(27-84)	
Gender			**0.3730**
Male	177(88.5)	171(85.5)	
Females	23(11.5)	29(14.5)	
Smoking Status			**<0.0001**
Smokers	161(80.5)	107(53.5)	
Non-Smokers	39(19.5)	93(46.5)	
Pack Years			**<0.0001**
Mean ± SD	80.0 ± 40.76	17.26 ± 12.895	
GOLD Severity			
I	7(3.5)		
II	74(37)		
III	87(43.5)		
IV	32(16)		
mMRC			
mMRC < 2	33(16.5)		
mMRC ≥ 2	167(83.5)		
CAT score			
CAT < 10	33(16.5)		
CAT ≥ 10	167(83.5)		
GOLD "ABCD"			
A	29(14.5)		
B	146(73)		
E	25(12.5)		
Duration of COPD			
< 2 years	83(41.5)		
2-5 years	51(25.5)		
5-10 years	31(15.5)		
>10 years	35(17.5)		

SD=Standard Deviation. p values were derived from the Pearson Chi-square test except for age and pack years; the student t-test was used for age and pack years. All p values are two-sided **p<0.05** was considered statistically significant.

Table 5.4: Distribution of demographic characteristics of COPD cases and controls.

The demographical characteristics considered for both cases and controls were age, gender, smoking status, pack-years, smoking index, GOLD severity, mMRC, CAT score, GOLD group and duration of COPD, mentioned in table 5.4. This study comprised 200 cases as well as controls. The average age of the patients was 58.54 ± 11.425 and 57.31 ± 7.59 in the case of controls. The distribution for age was insignificant (p=0.2055). The study included 177 males and 23 females in patients while 171 males and 29 females in controls. The p-value was calculated to be 0.373, indicating that gender is an independent factor. In this study, 161 were smokers, and 39 were non-smoker patients, while in controls, 107 were smokers, and 93 were non-smokers. Smoking was a significant risk factor for the prognosis of COPD ($p< 0.0001$). Mean pack years among COPD patients were significantly higher (80.0 ± 40.76) than that of controls (17.26 ± 12.895).

GOLD severity calculated based on FEV value was divided into four groups – I, II, III, and IV, where I am the least severe, and IV is the most severe. For cases, the distribution was 7 in group I, 74 in group II, 87 in Group III, and 32 in Group IV. No such distribution was done for controls. According to GOLD guidelines, the distribution for mMRC consisted of diving into groups of mMRC < 2 and mMRC ≥ 2. The group mMRC < 2 comprised 32 cases, and the group mMRC ≥ 2 comprised 168 cases. mMRC grading is not applicable for controls. Similar to mMRC, the CAT score was divided into two groups- CAT <10 and CAT≥10, according to GOLD guidelines. The group CAT <10 comprised 33 and 167 in CAT≥10. GOLD ABE parameter is a combinatorial method of grading the severity of COPD. Group A comprised 29 cases, 146 in group B and 25 in group E. The duration of COPD was also classified among COPD subjects, divided into four groups, i.e., <2 years,2-5 years, 5-10 years, and >10 years. The majority of the cases in this study had COPD for less than two years, which comprised 83 patients, 51 patients who had COPD for 2-5 years, 31 patients who had this disease for 5-10 years, and 35 patients who had COPD for more than ten years.

5.4.1 GOLD severity distribution among COPD cases

GOLD severity of grouping COPD patients according to their severity is based on the FEV1 value post-bronchodilation. The pie chart shows the severity group distribution among the patients in the present study. GOLD I being the least severe, having FEV1 ≥80% predicted, comprised 3% (7 out of 200) of the total cases. GOLD II has moderate severity as 50% ≤ FEV1 < 80% predicted, comprised of 37% cases (74 out of 200). GOLD III has severe severity as 30% ≤ FEV1 < 50%, comprised of 44% (87 out of 200). GOLD IV has severe severity as FEV1 <30% predicted, comprised of 16% (32 out of 200).

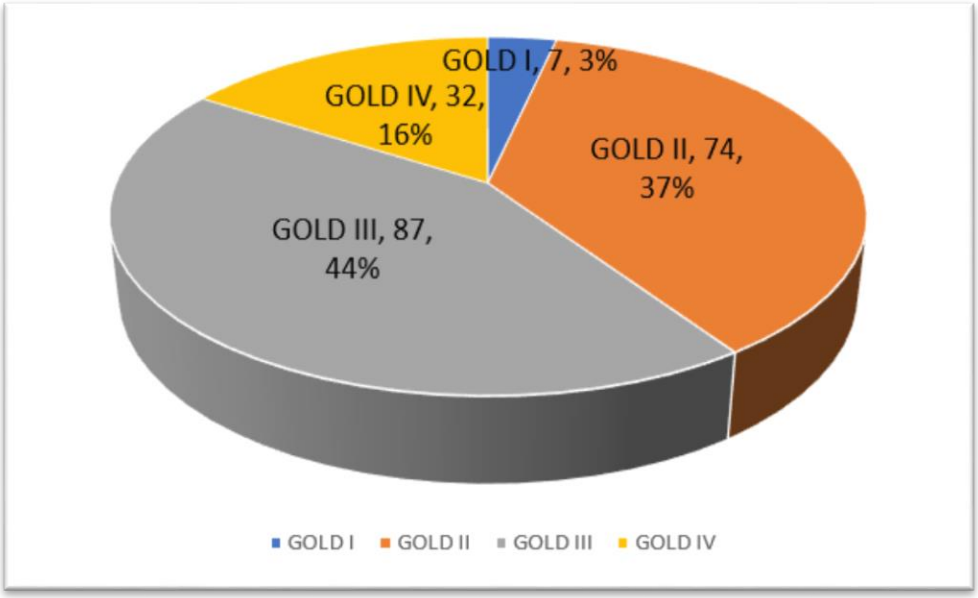


Fig 20: Pie illustration depicting GOLD severity distribution among COPD patients.

5.4.2 mMRC distribution among COPD cases

Modified Medical Research Council (mMRC) severity is based on the degree od breathlessness in respiratory diseases, especially for COPD cases. According to GOLD guidelines, mMRC is categorised into two groups - mMRC<2 and mMRC ≥2. In this study, the mMRC<2 group comprised 16% COPD cases (33 out of 200) and 84% COPD cases (167 out of 200) in mMRC ≥2.

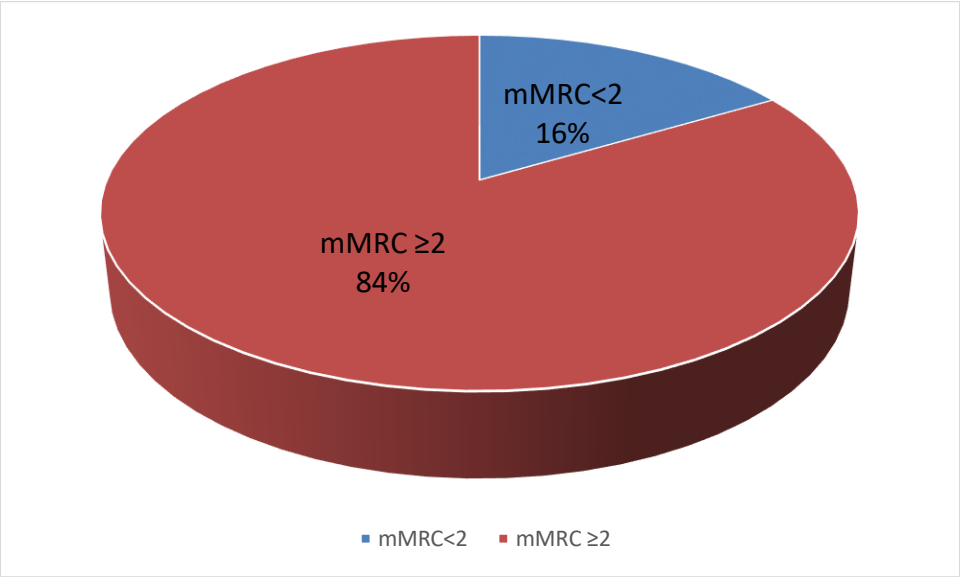


Fig 21: Pie illustration depicting mMRC distribution among COPD patients.

5.4.3 CAT score distribution among COPD cases

COPD Assessment Test (CAT) is an eight-listed specification questionnaire that evaluates the patient's health status. The score ranges from 0 to 40, where the higher the severity of the disease the score assigned. According to GOLD guidelines, CAT<10 and CAT ≥10 are the two groups assigned. In this study, the CAT<10 group comprised 16% COPD cases (33 out of 200) and 84% COPD cases (167 out of 200) in CAT ≥10.

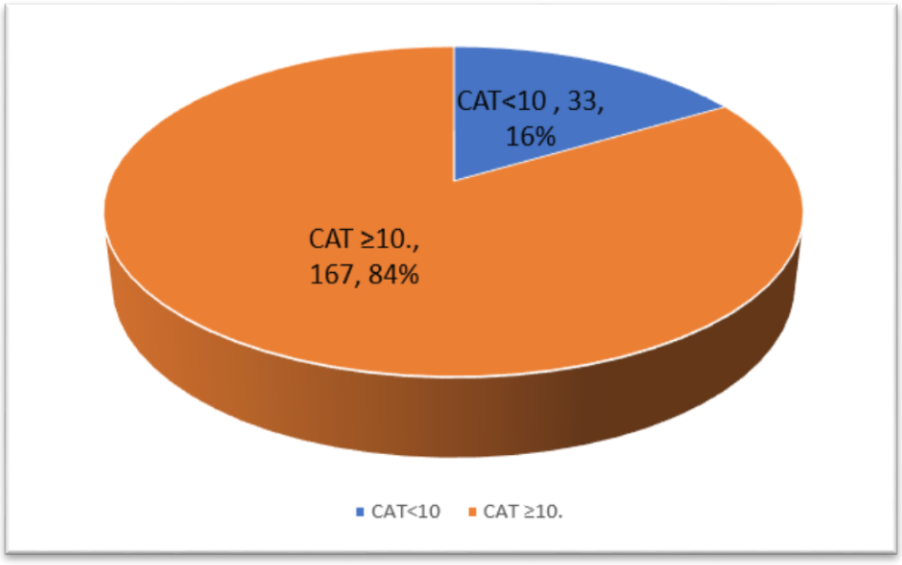


Fig 22: Pie illustration depicting CAT score distribution among COPD patients.

5.4.4 GOLD Group ABE distribution among COPD cases

GOLD group ABE is the grading of severity of the disease simply from a spirometric grading system to a combinatorial method based on the level of symptoms (mMRC and CAT), the severity of airflow obstruction (Gold severity FEV) and the frequency of previous worsening symptoms. GOLD A comprised 29 (14%) patients with few symptoms and a low risk of exacerbations; GOLD B formed 146 (73%) COPD subjects with more symptoms and a low risk of exacerbations. GOLD E includes 22 (11%) cases with more symptoms and an increased risk of exacerbations, as represented in the pie chart below.

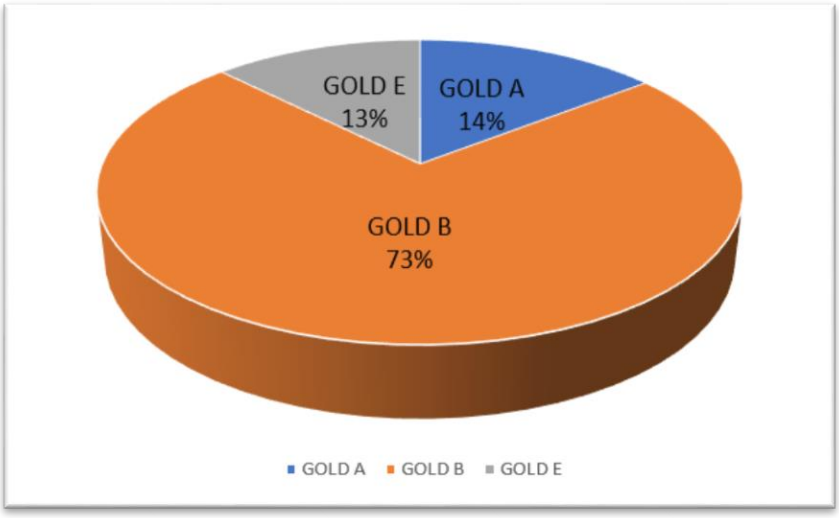


Fig 23: Pie illustration depicting GOLD group ABE distribution among COPD patients.

5.4.5 Duration of COPD distribution among cases

The duration of COPD was also grouped among COPD cases, and four divisions were made, i.e., <2 years,2-5 years, 5-10 years, and >10 years. For less than two years, which comprised 83 (41.5%) patients, 51 (25.5%) patients who had COPD for 2-5 years, 31 (15.5%) patients who had this disease for 5-10 years, and 35 (17.5%) patients had COPD for more than ten years. The majority of the cases had COPD for <2 years.

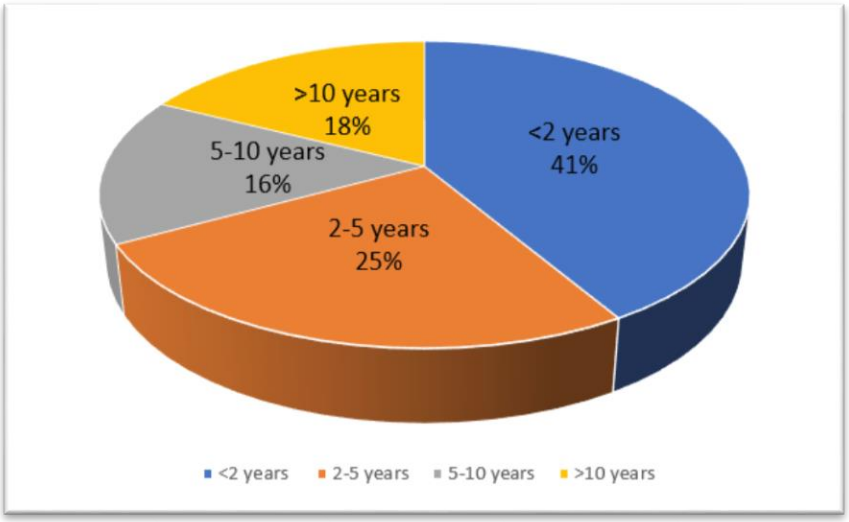


Fig 24: Pie illustration depicting the duration of COPD distribution among patients.

5.5 Results of *SOD1* gene polymorphism with COPD and its clinical parameters

5.5.1 Distribution and Association of SOD1 Polymorphism with risk associated with COPD.

The relationship between 50 bp Ins/Del polymorphism was investigated for COPD susceptibility. In the co-dominant model, Ins/Del heterozygous genotype polymorphism was detected in 28.5% of cases in COPD patients and 25.0% in controls. No significant association for COPD risk was established between cases and controls as OR=1.27, 95% CI= 0.817-1.99, p=0.28. Even after adjustment with covariates like age, gender and smoking, no significant association was found. The mutant polymorphic genotype of Del/Del was detected in 5.5% of individuals in COPD patients and 1.0% in controls. A significant associated contribution to the risk for COPD was found as an increase of approximately 5.5 folds in OR was observed compared to homozygous variants (OR=6.16, 95% CI=1.34-28.33, p=0.019). After adjustment with covariates, a slight but further increase in OR was observed (OR=6.56, 95% CI=1.35-31.88, p=0.0197). COPD risk was found to be of no significance in the dominant model. The recessive model was found to have approximately four-folds higher risk factor when compared to homozygous and heterozygous variants, with significant association (OR=5.76, 95% CI=1.26-26.33, p=0.0239). After adjustment with covariates, a slight decrease in the OR was observed, but the results were still significant and of high-risk factor. (OR=4.869, 95% CI=1.18-19.97, p=0.0279).

OVERALL						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	148 (74.0)	132 (66.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	50 (25.0)	57 (28.5)	1.27 (0.817-1.99)	0.28	1.27 (0.79-2.03)	0.318
Del/Del	2 (1.0)	11 (5.5)	6.16 (1.34-28.33)	0.019	6.56 (1.35-31.88)	0.0197
Dominant	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	148 (74.0)	132 (66.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	52 (26.0)	68 (34.0)	1.46 (0.95-2.25)	0.0815	1.464 (0.93-2.30)	0.0987
Recessive	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	198 (99.0)	189 (94.5)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	2 (1.0)	11 (5.5)	5.76 (1.26-26.33)	0.0239	4.869 (1.18-19.97)	0.0279

Table 5.5.1: Overall distribution and association of *SOD*1 polymorphism with COPD.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.2 Association of SOD1 polymorphism and CAT score for COPD risk

COPD Assessment Test (CAT) is an eight-listed specification questionnaire that evaluates the patient's health status. The score ranges from 0 to 40, where the higher the severity of the disease, more the score.

5.5.2.1 Comparing CAT score <10 and CAT score≥10 (within the group)

The association between *SOD1* polymorphism and CAT score was evaluated by comparing CAT score <10 and CAT score≥10, where CAT score <10 was considered as control. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though, heterozygous in the codominant model and dominant showed a slight increase in the risk factor for COPD susceptibility.

CAT score <10 and CAT score≥10						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=33**	**n (%) N=167**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	23 (69.69)	109 (65.26)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	7 (21.21)	50 (29.94)	1.50 (0.60-3.74)	0.884	1.59 (0.62-4.06)	0.3327
Del/Del	3 (9.09)	8 (4.79)	0.56 (0.13-2.28)	0.421	0.59 (0.14-2.53)	0.485
Dominant	**n (%) N=33**	**n (%) N=167**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	23(69.69)	109 (65.26)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	10 (30.3)	58 (34.73)	1.22 (0.54-3.74)	0.624	1.28 (0.56-2.95)	0.5509
Recessive	**n (%) N=33**	**n (%) N=167**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	30 (90.9)	159 (95.21)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	3 (9.09)	8 (4.79)	0.50 (0.126-2.0)	0.330	0.72(0.35-1.48)	0.386

Table 5.5.2.1: Association of *SOD1* polymorphism and CAT score for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.2.2 Comparing CAT score <10 and CAT score≥10 with controls

The association between *SOD1* polymorphism and CAT score was evaluated by comparing CAT score <10 and CAT score≥10 with controls, respectively. For the group CAT score <10 and controls, in the codominant model, heterozygous polymorphic genotypes showed no association in contribution to the risk of COPD susceptibility. But in recessive genotypes in the codominant model, a significant nine-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=9.65, 95% CI=0.36-2.22, p=0.015). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a twenty-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=20.05, 95%CI=1.99-201.1, p=0.01). The dominant model produced no significant association for the risk of COPD susceptibility. In the recessive model, a significant, approximately ten-fold increase in the risk of COPD susceptibility was observed (OR=9.90, 95% CI=1.58-61.7, p=0.0141). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status (AOR=4.43, 95%CI=1.46-13.44, p=0.008)

For the group CAT score≥10 and controls, in the codominant model, heterozygous polymorphic genotypes showed no association in contribution to the risk of COPD susceptibility. But in recessive genotypes in the codominant model, a significant five-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=5.43, 95% CI=1.13-26.0, p=0.034). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing an eight-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=8.19, 95%CI=1.25-53.63, p=0.028). The dominant model produced no significant association for risk of COPD susceptibility, but a slight susceptibility was observed (AOR=1.71, 95% CI=1.02-2.86, p=0.039). In the recessive model, a significant approximately five-folds approximate increase in the risk of COPD susceptibility was observed (OR=4.98, 95% CI=1.04-23.78, p=0.044). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing an approximate seven-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=6.92, 95%CI=1.08-44.19, p=0.04).

CAT score <10 and controls						
Polymorphism						
SOD1	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=33**				
Ins/Ins	148 (74.0)	23 (69.69)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	50 (25.0)	7 (21.21)	0.90(0.36-2.22)	0.821	0.77 (0.28-2.14)	0.626
Del/Del	2 (1.0)	3 (9.09)	9.65 (1.52-60.9)	0.015	20.05 (1.99-201.1)	0.010
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=33**				
Ins/Ins	148 (74.0)	23(69.69)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	52 (26.0)	10 (30.3)	1.23 (0.55-2.77)	0.60	1.17 (0.48-2.84)	0.727
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=33**				
Ins/Ins + Ins/Del	198 (99.0)	30 (90.9)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	2 (1.0)	3 (9.09)	9.90 (1.58-61.7)	0.0141	4.43(1.46-13.44)	0.008
CAT score≥10 and controls						
Polymorphism						
SOD1	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=167**				
Ins/Ins	148 (74.0)	109 (65.26)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	50 (25.0)	50 (29.94)	1.24 (0.79-1.94)	0.333	1.52 (0.90-2.58)	0.116
Del/Del	2 (1.0)	8 (4.79)	5.43 (1.13-26.0)	0.034	8.19 (1.25-53.63)	0.028
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=167**				
Ins/Ins	148 (74.0)	109 (65.26)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	52 (26.0)	58 (34.73)	1.51 (0.96-2.37)	0.069	1.71 (1.02-2.86)	0.039
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=167**				
Ins/Ins + Ins/Del	198 (99.0)	159 (95.21)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	2 (1.0)	8 (4.79)	4.98 (1.04-23.78)	0.044	6.92(1.08-44.19)	0.04

Table 5.5.2.2: Association of *SOD1* polymorphism with CAT score and controls for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant.

5.5.3 Association of SOD1 polymorphism and age for COPD risk

5.5.3.1 Comparing age below average with controls

The average age for both cases and controls was 57 years. The association between *SOD1* polymorphism and age below average was evaluated by comparing patients with COPD having age below average and controls with age below average. For none of the models, codominant, dominant and recessive, except for the dominant model before adjustment, showed no association for the risk of COPD susceptibility was found to be significant. In the dominant model before adjustment, an approximately two-fold increase in the risk factor was observed compared to the controls (OR=1.98, 95% CI=1.04-3.77, p=0.035). Though homozygous recessive polymorphic genotypes of codominant and recessive models showed an approximately four-fold increase in the risk factor compared with the controls, the results were insignificant.

Age below average with controls						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	72 (75.78)	52 (61.17)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	21 (22.1)	26 (30.58)	1.71 (0.87-3.37)	0.18	1.63 (0.75-3.53)	0.20
Del/Del	2 (2.1)	7 (8.23)	4.84 (0.96-24.28)	0.054	5.14 (0.70-37.6)	0.10
Dominant	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	72 (75.78)	52 (61.17)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	23 (24.21)	33 (38.82)	1.98 (1.04-3.77)	0.035	1.91 (0.91-3.97)	0.083
Recessive	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	93 (97.89)	78 (91.76)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	2 (2.1)	7 (8.23)	4.17 (0.84-20.67)	0.08	4.40(0.68-28.45)	0.119

Table 5.5.3.1: Association of *SOD1* polymorphism with age below average for both cases and controls for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant.

5.5.3.2 Comparing age above average with controls

The association between *SOD1* polymorphism and CAT score was evaluated by comparing cases and controls age groups above average. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though homozygous recessive polymorphic variants in the codominant and recessive models showed an approximate nine-fold increase in the risk factor before adjustment, the data obtained was highly insignificant.

Age above average with controls						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=105**	**n (%) N=115**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	76 (72.38)	80 (69.56)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	29 (27.61)	31 (26.95)	1.05 (0.55-1.84)	0.95	1.21 (0.57-2.59)	0.60
Del/Del	0 (0.0)	4 (3.47)	8.55 (0.45-161.5)	0.15	-	0.99
Dominant	**n (%) N=105**	**n (%) N=115**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	76 (72.38)	80 (69.56)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	29 (27.61)	35 (30.43)	1.14 (0.63-2.05)	0.64	1.32 (0.62-2.79)	0.45
Recessive	**n (%) N=105**	**n (%) N=115**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	105 (100)	111 (96.52)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	0 (2.1)	4 (3.47)	9.32 (0.49-175.1)	0.13	-	0.99

Table 5.5.3.2: Association of *SOD1* polymorphism with age above average for both cases and controls for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.4 Association of SOD1 polymorphism and mMRC for COPD susceptibility

According to GOLD guidelines, the mMRC criterion for grading severity among COPD patients is divided into four groups- 1, 2, 3 and 4. The greater the number of the group assigned to a patient, the higher the severity.

5.5.4.1 Comparing mMRC groups 1 and 2 with 3 and 4

The association between *SOD1* polymorphism and mMRC groups 1 and 2 with 3 and 4 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. Groups 1 and 2 are less severe when compared to Groups 3 and 4, Groups 1 and 2 are treated as controls and Groups 3 and 4 are more severe, so they are treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant, and no positive risk factor was identified as no rise in OR contributing to the risk of COPD susceptibility was detected when compared with controls.

mMRC 1&2 with 3&4						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	86 (63.23)	46 (71.87)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	41 (30.14)	16 (25.0)	0.72 (0.36-1.43)	0.36	0.81 (0.40-1.65)	0.57
Del/Del	9 (6.61)	2 (3.12)	0.41 (0.08-2.00)	0.27	0.44 (0.08-2.22)	0.32
Dominant	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	86 (63.23)	46 (71.87)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	50 (36.76)	18 (28.12)	0.67 (0.35-1.28)	0.23	0.74 (0.37-1.46)	0.39
Recessive	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	127 (93.38)	62 (96.87)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	9 (6.61)	2 (3.12)	0.45 (0.09-2.17)	0.32	0.46 (0.09-2.35)	0.35

Table 5.5.4.1: Association of *SOD1* polymorphism with mMRC group 1 & 2 with group 3 &4 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **$p<0.05$** was considered to be statistically significant

5.5.4.2 Comparing mMRC group 2 with 3

The association between *SOD1* polymorphism and mMRC group 2 with 3 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. As group 2 is less severe when compared to group 3, group 2 are treated as controls and group 3 is more severe, treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant, and no positive risk factor was identified as no rise in OR contributing to the risk of COPD susceptibility was detected when compared with controls.

mMRC 2 with 3						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=103**	**n (%) N=61**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	61 (63.23)	45 (71.87)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	35 (30.14)	14 (25.0)	0.54 (0.26-1.12)	0.36	0.64 (0.29-1.37)	0.25
Del/Del	7 (6.61)	2 (3.12)	0.38 (0.07-1.95)	0.10	0.42 (0.08-2.21)	0.30
Dominant	**n (%) N=103**	**n (%) N=61**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	61 (63.23)	45 (71.87)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	42 (36.76)	16 (28.12)	0.51 (0.25-1.03)	0.06	0.60 (0.29-1.24)	0.17
Recessive	**n (%) N=103**	**n (%) N=61**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	96 (93.38)	59 (96.87)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	7 (6.61)	2 (3.12)	0.46 (0.09-2.31)	0.34	0.48 (0.09-2.53)	0.39

Table 5.5.4.1: Association of *SOD1* polymorphism with mMRC group 2 with group 3 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.5 Relationship of SOD1 Polymorphism and GOLD Severity for COPD Risk

According to GOLD guidelines, the GOLD severity criterion for grading severity among COPD patients based on FEV1 value is divided into four groups- 1, 2, 3 and 4. The greater the number of the group assigned to a patient, the higher the severity showing more significant obstruction.

5.5.5.1 Comparing GOLD severity 1 and 2 with 3 and 4.

The association between *SOD1* polymorphism and GOLD severity 1 and 2 with 3 and 4 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. Groups 1 and 2 are less severe when compared to Groups 3 and 4, Groups 1 and 2 are treated as controls and Groups 3 and 4 are more severe, so they are treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant, and no positive risk factor was identified as no rise in OR contributing to the risk of COPD susceptibility was detected when compared with controls.

GOLD severity 1&2 with 3&4						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	52 (64.19)	80 (67.22)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	24 (29.62)	33 (27.73)	0.89 (0.47-1.68)	0.72	0.92 (0.48-1.75)	0.81
Del/Del	5 (6.17)	6 (5.04)	0.78 (0.22-2.68)	0.69	0.80 (0.23-2.78)	0.73
Dominant	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	52 (64.19)	80 (67.22)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	29 (35.8)	39 (32.77)	0.87 (0.48-1.58)	0.65	0.90 (0.49-1.64)	0.73
Recessive	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	76 (93.82)	113 (94.95)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	5 (6.17)	6 (5.04)	0.80 (0.23-2.73)	0.73	0.84 (0.24-2.89)	0.78

Table 5.5.4.1: Association of *SOD1* polymorphism with mMRC group 1 & 2 with group 3 &4 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.5.2 Comparing GOLD severity 2 with 3.

The association between *SOD1* polymorphism and GOLD severity group 2 with 3 was evaluated by comparing cases and controls. In this case, the severity of obstruction in airflow among COPD patients is compared. As group 2 is less severe when compared to group 3, group 2 are treated as controls and group 3 is more severe, treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant, and no significant rise in the risk factor was identified as no rise in OR contributing to the risk of COPD susceptibility was detected, when compared with controls.

GOLD severity 2 with 3						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=74**	**n (%) N=87**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	49 (66.21)	58 (66.67)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	20 (27.02)	23 (26.43)	0.97 (0.47-1.97)	0.93	1.01 (0.49-2.09)	0.96
Del/Del	5 (6.75)	6 (6.89)	1.01 (0.29-3.52)	0.98	1.02 (0.29-3.58)	0.96
Dominant	**n (%) N=74**	**n (%) N=87**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	49 (66.21)	58 (66.67)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	25 (33.78)	29 (33.34)	0.98 (0.5-1.88)	0.95	1.01 (0.52-1.98)	0.95
Recessive	**n (%) N=74**	**n (%) N=87**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	69 (93.24)	81 (93.10)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	5 (6.75)	6 (6.89)	1.02 (0.29-3.49)	0.97	1.06 (0.30-3.69)	0.91

Table 5.5.5.2: Association of *SOD1* polymorphism with GOLD severity group 2 with group 3 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.6 Relationship of SOD1 polymorphism and GOLD group ABE for COPD risk

GOLD group ABE is the grading of severity of the disease simply from a spirometric grading system to a combinatorial method based on the level of symptoms (mMRC and CAT), the severity of airflow obstruction (Gold severity FEV) and the frequency of previous worsening symptoms.

5.5.6.1 Comparing GOLD Group A with B and E

The association between *SOD1* polymorphism and GOLD group A with B and E was evaluated by comparing cases and controls. In this case, group A is less severe when compared to groups B and E; group A is treated as controls and group B and E being more severe, are treated as cases. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though homozygous recessive polymorphic variants in the codominant model showed an approximately two-fold increase in the risk factor compared with the controls, the data obtained was highly insignificant.

GOLD group A with B & E						
Polymorphism ***SOD1***	**Controls**	**Cases**				
Codominant	**n (%) N=29**	**n (%) N=171**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	22 (75.86)	110 (64.32)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	6 (20.68)	51 (29.82)	1.70 (0.64-4.44)	0.27	1.77 (0.65-4.77)	0.25
Del/Del	1 (3.44)	10 (5.84)	2.00 (0.24-16.43)	0.51	2.03 (0.26-19.79)	0.44
Dominant	**n (%) N=29**	**n (%) N=171**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	22 (75.86)	110 (64.32)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	7 (24.13)	61 (35.67)	1.74 (0.70-4.31)	0.22	1.85 (0.72-4.71)	0.19
Recessive	**n (%) N=29**	**n (%) N=171**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	28 (96.55)	161 (94.15)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	1 (3.44)	10 (5.84)	1.73 (0.21-14.12)	0.60	1.97 (0.23-16.56)	0.53

Table 5.5.6.1: Association of *SOD1* polymorphism with GOLD group A with B and E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.6.2 Comparing GOLD Group B with E

In this case, group B is less severe when compared to group E; group B is treated as a control and group E, being more severe, is treated as a case. Heterozygous polymorphic variants did not show any significant association for contributing to risk factors for COPD susceptibility. But recessive genotypes in the codominant model, a significant four-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=4.22, 95% CI=1.06-16.73, p=0.04). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a five-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=5.04, 95%CI=1.17-21.55, p=0.02). Dominant model before adjustment, a significant three-fold increase in the risk factor for COPD susceptibility was observed (OR=3.10, 95% CI=1.06-9.03, p=0.03). Though after adjustment, the data was found to be insignificant. In the recessive model, a significant approximately four-fold increase in the risk of COPD susceptibility was observed (OR=4.44, 95% CI=0.21-14.12, p=0.02). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a five-fold increase in the risk factor (AOR=5.06, 95%CI=121-21.09, p=0.02).

GOLD group B with E						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=146**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	95 (65.06)	15 (60.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	45 (30.82)	6 (24.0)	0.84 (0.30-2.32)	0.74	1.03 (0.36-2.92)	0.94
Del/Del	6 (4.1)	4 (16.0)	4.22 (1.06-16.73)	0.04	5.04 (1.17-21.55)	0.02
Dominant	**n (%) N=146**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	95 (65.06)	15 (60.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	51 (34.93)	10 (40.0)	3.10 (1.06-9.03)	0.03	1.55 (0.72-4.71)	0.33
Recessive	**n (%) N=146**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	140 (95.89)	21 (84.0)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	6 (4.1)	4 (16.0)	4.44 (0.21-14.12)	0.02	5.06 (1.21-21.09)	0.02

Table 5.5.6.2: Association of *SOD1* polymorphism with GOLD group B with E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.6.3 Comparing GOLD Group A with E

The association between *SOD1* polymorphism and GOLD group A with E was evaluated by comparing cases and controls. In this case, group A is less severe when compared to group E. Group A is treated as controls, and group E, being more severe, is treated as cases. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though homozygous recessive polymorphic variants in the codominant model, the dominant and recessive models showed six-fold, two-fold and seven-fold increases in the risk factor, respectively, when compared with the controls, but the data obtained was highly insignificant.

GOLD group A with E						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=29**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	22 (75.86)	15 (60.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	6 (20.68)	6 (24.0)	1.46 (0.39-5.42)	0.56	1.36 (0.30-6.12)	0.68
Del/Del	1 (3.44)	4 (16.0)	5.86 (0.59-57.78)	0.12	6.85 (0.54-86.20)	0.13
Dominant	**n (%) N=29**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	22 (75.86)	15 (60.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	7 (24.13)	10 (40.0)	2.09 (0.65-6.73)	0.21	2.20 (0.55-8.69)	0.25
Recessive	**n (%) N=29**	**n (%) N=25**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	28 (96.55)	21 (84.0)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	1 (3.44)	4 (16.0)	5.33 (0.55-51.27)	0.14	7.70 (0.61-96.3)	0.11

Table 5.5.6.3: Association of *SOD1* polymorphism with GOLD group A with E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.7 Association of SOD1 polymorphism with symptoms for COPD risk

5.5.7.1 Breathlessness

Breathlessness, the most crucial symptomatic factor, showed the highest association among patients, thus contributing to the highest risk of COPD pathogenesis. In our study of two-hundred patients, only four patients among two hundred were asymptomatic. Therefore, this concludes that breathlessness is highly associated with COPD prognosis.

5.5.7.2 Cough

The association between *SOD1* polymorphism and cough was evaluated by comparing cases and controls. Patients with no cough (asymptomatic) were treated as controls, while patients with a cough were treated as cases. For none of the models, codominant, dominant and recessive obtained any association for the risk of COPD susceptibility; thus, it was concluded to be insignificant.

Cough						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=143**	**n (%) N=57**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	97 (67.83)	35 (61.40)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	36 (25.17)	21 (36.84)	1.61 (0.83-3.13)	0.15	1.61 (0.80-3.23)	0.17
Del/Del	10 (6.99)	1 (1.75)	0.27 (0.03-2.24)	0.22	0.23 (0.02-2.01)	0.18
Dominant	**n (%) N=143**	**n (%) N=57**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	97 (67.83)	35 (61.40)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	46 (32.16)	22 (38.59)	1.32 (0.70-2.50)	0.38	1.30 (0.66-2.55)	0.44
Recessive	**n (%) N=143**	**n (%) N=57**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	133 (93.0)	56 (98.24)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	10 (6.99)	1 (1.75)	0.23 (0.02-1.89)	0.17	0.20 (0.02-1.69)	0.14

Table 5.5.7.2: Association of *SOD1* polymorphism with cough.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.5.7.3 Expectoration

The association between *SOD1* polymorphism and expectoration was evaluated by comparing cases and controls. Patients with no expectoration (asymptomatic) were treated as controls, while patients with expectoration were treated as cases. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant.

Expectoration						
Polymorphism *SOD1*	**Controls**	**Cases**				
Codominant	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	103 (68.67)	29 (58.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del	37 (24.67)	20 (40.0)	1.91 (0.97-3.79)	0.06	1.96 (0.94-4.06)	0.06
Del/Del	10 (6.67)	1 (2.0)	0.35 (0.04-2.89)	0.33	0.32 (0.03-2.75)	0.29
Dominant	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins	103 (68.67)	29 (58.0)	1.00 (Reference)	-	1.00 (Reference)	-
Ins/Del + Del/Del	47 (31.34)	21 (42.0)	1.58 (0.82-3.06)	0.16	1.61 (0.79-3.25)	0.18
Recessive	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
Ins/Ins + Ins/Del	140 (93.34)	49 (98.0)	1.00 (Reference)	-	1.00 (Reference)	-
Del/Del	10 (6.67)	1 (2.0)	0.21 (0.02-1.68)	0.14	0.24 (0.02-2.15)	0.20

Table 5.5.7.3: Association of *SOD1* polymorphism with expectoration.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant.

5.6 Results of *Catalase* gene polymorphism with COPD and its clinical parameters

5.6.1 Distribution and Association of Catalase Polymorphism with risk associated with COPD.

The relationship between 262 C>T polymorphism was investigated for COPD susceptibility. In the co-dominant model, 262 C>T heterozygous genotype polymorphism was detected in 38.5% of COPD patients and 34.5% of controls. No significant association for COPD risk was established between cases and controls as OR=95, 95% CI= 0.62-1.44, p=0.81. Even after adjustment with covariates like age, gender and smoking, no significant association was found. The mutant polymorphic genotype of T/T was detected in 10.0% of individuals in COPD patients and 2.5% in controls. A significant associated contribution towards the risk for COPD was found as an increase of approximate four-folds in OR was observed compared to homozygous variants (CC) (OR=4.25, 95% CI=1.54-11.71, p=0.005). After adjustment with covariates, a slight but further increase in OR was observed (OR=4.66, 95% CI=1.62-13.33, p=0.004). COPD risk was found to be of no significance in the dominant model. The recessive model was found to have approximately four-fold higher risk factor when compared to homozygous plus heterozygous variants (CC+CT), with significant association (OR=4.34, 95% CI=1.59-11.78, p=0.004). After adjustment with covariates, a slight increase in the OR was observed, but the results were still significant and of high-risk factor. (OR=4.62, 95% CI=1.63-13.11, p=0.003).

OVERALL						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=200**				
CC	118 (59.0)	111 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT	77 (38.5)	69 (34.5)	0.95 (0.62-1.44)	0.81	1.02 (0.65-1.58)	0.92
TT	5 (2.5)	20 (10.0)	4.25 (1.54-11.71)	0.005	4.66 (1.62-13.33)	0.004
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=200**				
CC	118 (59.0)	111 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	83 (41.5)	89 (44.5)	1.13 (0.76-1.69)	0.51	1.25 (0.82-1.90)	0.29
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=200**				
CC + CT	195 (97.5)	180 (90.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	5 (2.5)	20 (10.0)	4.34 (1.59-11.78)	0.004	4.62 (1.63-13.11)	0.003

Table 5.6.1: Overall distribution and association of *Catalase* polymorphism with COPD.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.2 Association of Catalase Polymorphism and CAT Score for COPD Risk

COPD Assessment Test (CAT) is an eight-listed specification questionnaire that evaluates the patient's health status. The score ranges from 0 to 40, where the higher the severity of the disease, more the score.

5.6.2.1 Comparing CAT score <10 and CAT score≥10 (within the group)

The association between *catalase* polymorphism and CAT score was evaluated by comparing CAT score <10 and CAT score≥10, where CAT score <10 was considered control. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though heterozygous in the codominant model and dominant showed a slight increase in the risk factor for COPD susceptibility, the data obtained were insignificant.

CAT score <10 and CAT score≥10						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%) N=32**	**n (%) N=168**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	20 (59.0)	91 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT	8 (38.5)	61 (34.5)	1.67 (0.69-4.04)	0.25	1.94 (0.77-4.86)	0.15
TT	4 (2.5)	16 (10.0)	0.87 (0.26-2.91)	0.83	1.02 (0.29-3.57)	0.96
Dominant	**n (%) N=32**	**n (%) N=168**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	20 (59.0)	91 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	12 (41.5)	77 (44.5)	1.41 (0.64-3.06)	0.38	1.67 (0.73-3.80)	0.21
Recessive	**n (%) N=32**	**n (%) N=168**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC + CT	28 (97.5)	152 (90.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	4 (2.5)	16 (10.0)	0.73 (0.22-2.36)	0.60	0.83 (0.25-2.81)	0.77

Table 5.6.2.1: Association of *Catlase* polymorphism and CAT score for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.2.2 Comparing CAT score <10 and CAT score≥10 with controls

The association between *Catalase* polymorphism and CAT score was evaluated by comparing CAT score <10 and CAT score≥10 with controls, respectively.

For the group CAT score <10 and controls, in the codominant model, heterozygous polymorphic genotypes showed no association in contribution to the risk of COPD susceptibility. But in recessive genotypes in the codominant model, a significant four-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=4.72, 95% CI=1.16-19.1, p=0.02). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a five-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=5.85, 95%CI=1.28-26.6, p=0.02). The dominant model produced no significant association for the risk of COPD susceptibility. In the recessive model, a significant approximate five-fold increase in the risk of COPD susceptibility was observed (OR=5.57, 95% CI=1.41-21.9, p=0.001). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing an approximate seven-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=6.84, 95%CI=1.42-32.8, p=0.01)

For the group CAT score≥10 and controls, in the codominant model, heterozygous polymorphic genotypes showed no association in contribution to the risk of COPD susceptibility. But in recessive genotypes in the codominant model, a significant four-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=4.14, 95% CI=1.46-11.7, p=0.007). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a four-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=4.09, 95%CI=1.37-12.1, p=0.011). The dominant model produced no significant association for the risk of COPD susceptibility, but a slight susceptibility was observed. In the recessive model, a significant approximately four-fold approximate increase in the risk of COPD susceptibility was observed (OR=4.10, 95% CI=1.47-11.45, p=0.007). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing an approximately four-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=4.09, 95%CI=1.39-12.05, p=0.01).

CAT score <10 and controls						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=32**				
CC	118 (59.0)	20 (59.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	77 (38.5)	8 (38.5)	0.61 (0.25-1.46)	0.26	0.64 (0.26-1.56)	0.33
TT	5 (2.5)	4 (2.5)	4.72 (1.16-19.1)	0.02	5.85 (1.28-26.6)	0.02
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=32**				
CC	118 (59.0)	20 (59.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	83 (41.5)	12 (41.5)	0.86 (0.40-1.86)	0.70	0.89 (0.40-2.01)	0.79
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=32**				
CC + CT	195 (97.5)	28 (97.5)	1.00 (Reference)	-	1.00 (Reference)	-
CC	5 (2.5)	4 (2.5)	5.57 (1.41-21.9)	0.01	6.84 (1.42-32.8)	0.01
CAT score≥10 and controls						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=168**				
CC	118 (59.0)	91 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT	77 (38.5)	61 (34.5)	1.02 (0.66-1.58)	0.90	1.09 (0.68-1.74)	0.69
TT	5 (2.5)	16 (10.0)	4.14 (1.46-11.7)	0.007	4.09 (1.37-12.1)	0.011
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=168**				
CC	118 (59.0)	91 (55.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	83 (41.5)	77 (44.5)	1.21 (0.80-1.84)	0.35	1.29 (0.83-2.02)	0.25
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=200	**N=168**				
CC + CT	195 (97.5)	152 (90.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	5 (2.5)	16 (10.0)	4.10 (1.47-11.45)	0.007	4.09 (1.39-12.05)	0.01

Table 5.6.2.2: Association of *Catalase* polymorphism with CAT score and controls for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.3 Association of Catalase Polymorphism and Age for COPD Risk

5.6.3.1 Comparing age below average with controls

The average age for both cases and controls was 57 years. The association between *catalase* polymorphism and age below average was evaluated by comparing patients with COPD having age below average and controls with age below average. In the codominant model, heterozygous polymorphic genotypes showed no association in contribution to the risk of COPD susceptibility. But in recessive genotypes in the codominant model, a significant eleven-fold increase in the risk of COPD susceptibility was observed when compared with wild type homozygous genotype (OR=11.08, 95% CI=1.44-96.5, p=0.02). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing a ten-fold increase in the risk factor when compared with wild-type homozygous genotype (AOR=10.79, 95%CI=1.07-108.0, p=0.04). The dominant model produced no significant association for the risk of COPD susceptibility. In the recessive model, a significant approximate eleven-fold increase in the risk of COPD susceptibility was observed (OR=11.13, 95% CI=1.37-89.8, p=0.02). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status (AOR=11.46, 95%CI=1.07-122.0, p=0.04).

Age below average cases with controls						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	59 (62.1)	45 (52.9)	1.00 (Reference)	-	1.00 (Reference)	-
CT	35 (36.8)	31 (36.4)	1.16 (0.62-2.15)	0.63	1.09 (0.54-2.19)	0.79
TT	1 (1.05)	9 (10.5)	11.80 (1.44-96.5)	0.02	10.79 (1.07-108.0)	0.04
Dominant	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	59 (62.1)	45 (52.9)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	36 (37.9)	40 (47.1)	1.45 (0.80-2.63)	0.21	1.35 (0.69-2.65)	0.37
Recessive	**n (%) N=95**	**n (%) N=85**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC + CT	94 (98.9)	76 (89.4)	1.00 (Reference)	-	1.00 (Reference)	-
CC	1 (1.05)	9 (10.5)	11.13 (1.37-89.8)	0.02	11.46 (1.07-122.0)	0.04

Table 5.6.3.1: Association of *Catalase* polymorphism with age below average for both cases and controls for COPD risk. **OR[1]**: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted

odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant.

5.6.3.2 Comparing age above average with controls

The association between *catalase* polymorphism and CAT score was evaluated by comparing cases and controls age groups above average. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. Though homozygous recessive polymorphic variants in codominant and recessive models showed an approximately two-fold increase in the risk factor, the data obtained was highly insignificant.

Age above average cases with controls						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=105	**N=115**				
CC	59 (56.2)	66 (57.4)	1.00 (Reference)	-	1.00 (Reference)	-
CT	42 (40.0)	38 (33.0)	0.81 (0.46-1.41)	0.45	0.74 (0.38-1.44)	0.38
TT	4 (3.80)	11 (9.56)	2.45 (0.74-8.13)	0.14	2.52 (0.69-9.13)	0.15
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=105	**N=115**				
CC	59 (56.2)	66 (57.4)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	46 (43.8)	49 (42.6)	0.95 (0.55-1.62)	0.85	0.91 (0.49-1.70)	0.77
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=105	**N=115**				
CC + CT	101 (96.2)	104 (90.4)	1.00 (Reference)	-	1.00 (Reference)	-
CC	4 (3.80)	11 (9.56)	2.67 (0.82-8.66)	0.10	2.88 (0.81-10.24)	0.10

Table 5.6.3.2: Association of *Catalase* polymorphism with age above average for both cases and controls for COPD risk.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.4 Association of Catalase Polymorphism and mMRC for COPD Susceptibility

5.6.4.1 Comparing mMRC groups 1 and 2 with 3 and 4

The association between *Catalase* polymorphism and mMRC groups 1 and 2 with 3 and 4 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. Groups 1 and 2 are less severe when compared to groups 3 and 4; groups 1 and 2 are treated as controls, and groups 3 and 4 are more severe, so they are treated as cases. This comparison is a comparison among the group. In the co-dominant model, heterozygous and homozygous recessive polymorphic variants obtained no significant association before adjustment. Though, after adjustment with age, gender and smoking, homozygous recessive remained insignificant, heterozygous polymorphic variants showed a significant association of two-folds in contributing to COPD susceptibility risk (AOR=2.01, 95%CI=1.01-3.98, p=0.045). The dominant model showed a significant association of approximately two-fold both before (OR=1.83, 95% CI=1.00-3.34, p=0.047) and after adjustment (AOR=2.10, 95%CI=1.10-3.99, p=0.02). The recessive model showed no significant association.

mMRC 1 & 2 with 3 & 4						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	82 (60.3)	29 (45.3)	1.00 (Reference)	-	1.00 (Reference)	-
CT	42 (30.8)	27 (42.2)	1.82 (0.95-3.45)	0.06	2.01 (1.01-3.98)	0.045
TT	12 (8.82)	8 (12.5)	1.88 (0.70-5.07)	0.20	2.34 (0.82-6.64)	0.11
Dominant	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	82 (60.3)	29 (45.3)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	54 (39.7)	35 (54.7)	1.83 (1.00-3.34)	0.047	2.10 (1.10-3.99)	0.02
Recessive	**n (%) N=136**	**n (%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC + CT	124 (91.2)	56 (87.5)	1.00 (Reference)	-	1.00 (Reference)	-
CC	12 (8.82)	8 (12.5)	1.47 (0.57-3.81)	0.42	1.81 (0.66-4.95)	0.24

Table 5.6.4.1: Association of *Catalase* polymorphism with mMRC group 1 & 2 with group 3 &4 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant.

5.6.4.2 Comparing mMRC group 2 with 3

The association between *Catalase* polymorphism and mMRC group 2 with 3 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. As group 2 is less severe when compared to group 3, group 2 are treated as controls and group 3 is more severe, treated as cases. This method of comparison is comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. In the co-dominant model, homozygous recessive polymorphic variants showed a two-fold increase in risk after adjustment, but the data obtained remained insignificant.

mMRC 2 with 3						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=103	**N=61**				
CC	62 (60.2)	29 (47.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT	33 (32.0)	25 (41.9)	1.61 (0.81-3.20)	0.16	1.73 (0.84-3.55)	0.13
TT	8 (7.76)	7 (11.4)	1.87 (0.61-5.65)	0.26	2.05 (0.65-6.46)	0.21
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=103	**N=61**				
CC	62 (60.2)	29 (47.5)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	41 (39.8)	32 (52.4)	1.66 (0.88-3.16)	0.11	1.80 (0.91-3.53)	0.08
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=103	**N=61**				
CC + CT	95 (92.2)	54 (88.5)	1.00 (Reference)	-	1.00 (Reference)	-
CC	8 (7.76)	7 (11.4)	1.53 (0.52-4.47)	0.42	1.59 (0.53-4.79)	0.40

Table 5.5.4.2: Association of *Catalase* polymorphism with mMRC group 2 with group 3 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **$p<0.05$** was considered to be statistically significant.

5.6.5 Relationship of Catalase Polymorphism and GOLD Severity for COPD Risk

According to GOLD guidelines, the GOLD severity criterion for grading severity among COPD patients based on FEV1 value is divided into four groups- 1, 2, 3 and 4. The greater the number of the group assigned to a patient, the higher the severity showing more significant obstruction.

5.6.5.1 Comparing GOLD severity 1 and 2 with 3 and 4.

The association between *catalase* polymorphism and GOLD severity 1 and 2 with 3 and 4 was evaluated by comparing cases and controls. In this case, severity among COPD patients is compared. Groups 1 and 2 are less severe when compared to groups 3 and 4; groups 1 and 2 are treated as controls, and groups 3 and 4 are more severe, so they are treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant, and no positive risk factor was identified as no rise in OR contributing to the risk of COPD susceptibility was detected when compared with controls.

Gold severity 1 & 2 with 3 & 4						
Polymorphism *Catalase*	**Controls**	**Cases**				
Codominant	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	40 (49.3)	71 (59.6)	1.00 (Reference)	-	1.00 (Reference)	-
CT	35 (43.2)	34 (28.6)	0.54 (0.29-1.01)	0.053	0.55 (0.29-1.02)	0.061
TT	6 (7.40)	14 (11.7)	1.31 (0.46-3.68)	0.60	1.41 (0.49-4.05)	0.52
Dominant	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	40 (49.3)	71 (59.6)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	41 (50.7)	48 (40.3)	0.65 (0.37-1.16)	0.15	0.67 (0.37-1.20)	0.18
Recessive	**n (%) N=81**	**n (%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC + CT	75 (92.6)	105 (88.2)	1.00 (Reference)	-	1.00 (Reference)	-
CC	6 (7.40)	14 (11.7)	1.66 (0.61-4.53)	0.31	1.82 (0.65-5.04)	0.24

Table 5.6.5.1: Association of *Catalase* polymorphism with mMRC group 1 & 2 with group 3 &4 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.5.2 Comparing GOLD severity 2 with 3.

The association between *catalase* polymorphism and GOLD severity group 2 with 3 was evaluated by comparing cases and controls. In this case, the severity of obstruction in airflow among COPD patients is compared. As group 2 is less severe when compared to group 3, group 2 are treated as controls and group 3 is more severe, treated as cases. This comparison is a comparison among the group. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant. In the recessive model, a two-fold rise in OR was observed after adjustment, but the values remained insignificant.

Gold severity 2 with 3						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=74	**N=87**				
CC	35 (47.3)	48 (55.1)	1.00 (Reference)	-	1.00 (Reference)	-
CT	33 (44.5)	27 (31.03)	0.59 (0.30-1.16)	0.13	0.62 (0.31-1.23)	0.17
TT	6 (8.10)	12 (13.8)	1.45 (0.49-4.26)	0.49	1.58 (0.52-4.75)	0.41
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=74	**N=87**				
CC	35 (47.3)	48 (55.1)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	39 (52.7)	39 (44.8)	0.72 (0.39-1.35)	0.31	0.77 (0.40-1.45)	0.42
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=74	**N=87**				
CC + CT	68 (91.9)	75 (86.2)	1.00 (Reference)	-	1.00 (Reference)	-
CC	6 (8.10)	12 (13.8)	1.81 (0.64-5.09)	0.25	1.99 (0.69-5.07)	0.19

Table 5.6.5.2: Association of *catalase* polymorphism with GOLD severity group 2 with group 3 for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.6 Relationship of Catalase Polymorphism and GOLD Group ABE for COPD Risk

GOLD group ABE is the grading of severity of the disease simply from a spirometric grading system to a combinatorial method based on the level of symptoms (mMRC and CAT), the severity of airflow obstruction (Gold severity FEV) and the frequency of previous worsening symptoms.

5.6.6.1 Comparing GOLD Group A with B and E

The association between *catalase* polymorphism and GOLD group A with B and E was evaluated by comparing cases and controls. In this case, group A is less severe compared to groups B and E, and group A is treated as controls and group B and E being more severe, are treated as cases. For none of the models, codominant, dominant and recessive, no association for the risk of COPD susceptibility was found to be significant.

Gold group A with B & E						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=171**				
CC	16 (55.1)	95 (55.56)	1.00 (Reference)	-	1.00 (Reference)	-
CT	9 (31.03)	60 (35.08)	1.12 (0.46-2.70)	0.79	1.29 (0.51-3.25)	0.57
TT	4 (13.79)	16 (9.35)	0.67 (0.19-2.27)	0.52	0.78 (0.21-2.86)	0.71
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=171**				
CC	16 (55.1)	95 (55.56)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	13 (44.8)	66 (38.59)	1.06 (0.47-2.39)	0.87	1.15 (0.50-2.68)	0.72
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=171**				
CC + CT	25 (86.2)	155 (90.64)	1.00 (Reference)	-	1.00 (Reference)	-
CC	4 (13.79)	16 (9.35)	0.64 (0.19-2.08)	0.46	0.73 (0.21-2.53)	0.62

Table 5.5.6.1: Association of *Catalase* polymorphism with GOLD group A with B and E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant.

5.6.6.2 Comparing GOLD Group B with E

In this case, group B is less severe when compared to group E; group B is treated as a control and group E, being more severe, is treated as a case. Heterozygous polymorphic variants did not show any significant association for contributing to risk factors for COPD susceptibility. But recessive genotypes in the codominant model, a significant approximate four-fold increase in the risk of COPD susceptibility was observed compared to wild type homozygous genotype (OR=3.86, 95% CI=1.11-13.4, p=0.033). Though the risk factor obtained after adjustment remained three times, the data obtained was insignificant. In the case dominant model, no significant association was obtained. In the recessive model, both before and after adjustment, the risk factor for COPD susceptibility remained three times, but the data obtained was insignificant.

Gold Group B with E						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=146	**N=25**				
CC	85 (58.2)	10 (40.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	50 (34.2)	10 (40.0)	1.70 (0.66-4.36)	0.13	1.62 (0.62-4.24)	0.32
TT	11 (7.53)	5 (20.0)	3.86 (1.11-13.4)	0.033	3.53 (0.98-12.72)	0.052
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=146	**N=25**				
CC	85 (58.2)	10 (40.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	61 (41.8)	15 (60.0)	2.09 (0.88-4.96)	0.09	2.01 (0.40-1.45)	0.11
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=146	**N=25**				
CC + CT	135 (92.4)	20 (80.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	11 (7.53)	5 (20.0)	3.06 (0.96-9.75)	0.057	3.04 (0.92-9.98)	0.066

Table 5.6.6.2: Association of *catalase* polymorphism with GOLD group B with E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.6.3 Comparing GOLD Group A with E

The association between *catalase* polymorphism and GOLD group A with E was evaluated by comparing cases and controls. In this case, group A is less severe when compared to group E; group A is treated as a control, and group E is more severe, treated as the case. Though the data obtained for all three models- co-dominant, dominant and recessive were insignificant, the risk factor for all the models remained approximately two – three folds high for each model.

Gold Group A with E						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=25**				
CC	16 (55.1)	10 (40.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	9 (31.03)	10 (40.0)	1.77 (0.53-5.89)	0.34	1.89 (0.48-7.41)	0.35
TT	4 (13.79)	5 (20.0)	2.00 (0.43-9.27)	0.37	3.21 (0.44-23.1)	0.24
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=25**				
CC	16 (55.1)	10 (40.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	13 (44.8)	15 (60.0)	1.84 (0.62-5.46)	0.26	2.27 (0.63-8.01)	0.20
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=29	**N=25**				
CC + CT	25 (86.2)	20 (80.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	4 (13.79)	5 (20.0)	1.56 (0.37-6.59)	0.54	2.76 (0.40-19.0)	0.30

Table 5.6.6.3: Association of *catalase* polymorphism with GOLD group A with E for COPD risk

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.6.7 Association of Catalase Polymorphism with Symptoms for COPD Risk

5.6.7.1 Breathlessness

Breathlessness, the most critical symptomatic factor, showed the highest association among patients, thus contributing to the highest risk of COPD pathogenesis. In our study of two-hundred patients, only four patients among two hundred were asymptomatic. Therefore, this concludes that breathlessness is highly associated with COPD prognosis.

5.6.7.2 Cough

The association between *catalase* polymorphism and cough was evaluated by comparing cases and controls. Patients with no cough (asymptomatic) were treated as controls, while patients with a cough were treated as cases. The heterozygous codominant and dominant models obtained no association for the risk of COPD susceptibility; thus, it was considered insignificant. In the case of homozygous recessive, codominant, and recessive models, though the data obtained was significant, the risk remained very low.

Cough						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=136	**N=57**				
CC	72 (52.9)	32 (56.1)	1.00 (Reference)	-	1.00 (Reference)	-
CT	45 (33.1)	24 (42.1)	1.20 (0.62-2.29)	0.58	1.43 (0.71-2.87)	0.31
TT	19 (13.9)	1 (1.80)	0.12 (0.015-0.92)	0.041	0.09 (0.012-0.81)	0.032
Dominant	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=136	**N=57**				
CC	72 (52.9)	32 (56.1)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	64 (47.1)	25 (43.9)	0.87 (0.47-1.63)	0.68	0.98 (0.50-1.90)	0.96
Recessive	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
	N=136	**N=57**				
CC + CT	127 (93.3)	56 (98.2)	1.00 (Reference)	-	1.00 (Reference)	-
CC	19 (13.9)	1 (1.80)	0.12 (0.015-0.91)	0.040	0.087 (0.01-0.73)	0.024

Table 5.6.7.2: Association of *catalase* polymorphism with cough.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking. A **p<0.05** was considered to be statistically significant

5.6.7.3 Expectoration

The association between *catalase* polymorphism and expectoration was evaluated by comparing cases and controls. Patients with no expectoration (asymptomatic) were treated as controls, while patients with expectoration were treated as cases. For codominant and dominant, no association for the risk of COPD susceptibility was found to be significant. Though in the recessive model, after adjustment, the data obtained was significant. But as the AOR obtained was very low, no risk factor in COPD susceptibility was concluded (AOR=0.11, 95%CI=0.013-0.96, p=0.045).

Expectoration						
Polymorphism						
Catalase	**Controls**	**Cases**				
Codominant	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	83 (55.34)	28 (56.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	48 (32.0)	21 (42.0)	1.29 (0.66-2.52)	0.44	1.50 (0.72-3.13)	0.27
TT	19 (12.67)	1 (2.00)	0.15 (0.02-1.21)	0.07	0.13 (0.015-1.19)	0.072
Dominant	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	83 (55.34)	28 (56.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT + CC	67 (44.67)	22 (44.0)	0.97 (0.51-1.85)	0.97	1.07 (0.53-2.15)	0.84
Recessive	**n (%) N=150**	**n (%) N=50**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC + CT	131 (87.34)	49 (98.0)	1.00 (Reference)	-	1.00 (Reference)	-
CC	19 (12.67)	1 (2.00)	0.14 (0.018-1.07)	0.059	0.11 (0.013-0.96)	0.045

Table 5.6.7.3: Association of *catalase* polymorphism with expectoration.

OR[1]: Crude odds ratio, **95% CI:** 95% confidence interval; **AOR[2]**: Adjusted odds ratio were evaluated by unconditional logistic regression and adjusted for age, gender, and smoking; A **p<0.05** was considered to be statistically significant

5.7 Combined effects of *SOD1* (50 bp Ins/Del) and *Catalase* (262 C>T) on the risk of COPD

This study evaluated the risk and contribution of COPD prognosis and the risk of different combinations of SNP-to-SNP interactions of SOD1 and catalase genotypes. The SNP combinations are classified based on risk factor grading and the number of affected alleles. Risk factor 0 indicates no alterations in the genotypes of the subjects. As no alterations are involved, only the wild-type combinations are included in this group. Thus, the only possible combination possible is Ins/Ins and C/C. 42.5% among controls and 38.0% among COPD patients were categorised in this group. Risk factor 1 indicates an alteration in either of the genes, thus possibly having a wild-type homozygous allele for one gene while heterozygous for the other. Ins/Del, C/C and Ins/Ins, C/T are the only possible combinations. 45.5% of controls and 35.5% of COPD patients were categorised in this group.

Risk factor 2 indicates two possible alterations in either one of the genes or one unaffected gene while the other with homozygous mutant allele. The only possible combinations are Ins/Del, C/T; Ins/Ins, T/T or Del/Del, C/C. 11% of controls and 21% of COPD patients were categorised in this group. Risk factor 3 indicates two possible alterations in one of the genes and heterozygous for the other gene and vice versa. The only possible combinations are Del/Del, C/T and Ins/Del, T/T. 1.00% among controls and 11% among COPD patients were categorised in this group.

There are no alterations in risk factor 0, so they are treated as a reference. Risk factor 1, having a single alteration, showed no association for the risk of COPD susceptibility was found to be significant. Risk factor 2, having two alterations, obtained a significant two-fold increase in the risk factor (OR=2.13, 95% CI=1.17-3.89, p=0.013). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing an approximately two-fold increase in the risk factor when compared with wild-type homozygous genotype of risk factor 1 (AOR=1.97, 95%CI=1.21-3.19, p=0.0056). Risk factor 3, having three alterations, obtained a significant six-fold increase in the risk factor (OR=6.15, 95% CI=1.32-28.63, p=0.020). The relationship remained even more substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status, showing more than a seven-fold increase in the risk factor when compared with wild-type homozygous genotype of risk factor 1 (AOR=7.71, 95%CI=1.58-37.5, p=0.0114).

As with the rise in the risk factor, there is a significant increase in the OR, suggesting the possibility of risk of COPD susceptibility with the increasing number of alterations in the genotypes.

No. of a risk allele	**Controls n (%) N=200**	**Cases n (%) N=200**	**OR[1] (CI)**	**P[3]**	**AOR[2] (CI)**	**P[4]**
0	85 (42.5)	76 (38.0)	1.00(Reference)	-	1.00(Reference)	-
1	91 (45.5)	71 (35.5)	0.87 (0.56-1.35)	0.872	0.94 (0.5-1.50)	0.82
2	22 (11.0)	42 (21.0)	2.13 (1.17-3.89)	0.013	1.97(1.21-3.19)	0.0056
3	2 (1.00)	11 (5.5)	6.15 (1.32-28.63)	0.020	7.71(1.58-37.5)	0.0114

1 Odds ratio, 95% confidence intervals, 2 adjusted odds ratio, 95% confidence interval calculated, and 3 corresponding p-values were calculated by logistic regression analysis 4 p-value after being adjusted 0-(C/C, Ins/Ins), 1- (C/C, Ins/Del), (C/T, Ins/Ins), 2- (C/C, Del/Del), (T/T, Ins/Ins), (C/T, Ins/Del), 3- (C/T, Del/Del), (T/T, Ins/Del)

Table 5.7: Combined effects of *SOD1* (50 bp Ins/Del) and *Catalase* (262 C>T) on the risk of COPD

CHAPTER 6

DISCUSSION

Due to the ever-depleting condition of the environment and nicotine addiction, it is evident that there would be a steady rise in the number of COPD cases all around the world. Our lungs are exposed to pollutants, allergens, irritants, noxious gases and particles, contributing to the risk factor to increase COPD susceptibility. Amir et al. predicted the future burden of COPD and stated that there might be a chance of about a 155% increase in the total cases due to the increasing air pollutants, ageing diseases, lifestyle changes and increasing population. Salvi *et al.,* in their epidemiological study for COPD trends prediction in India, clearly mentioned that there should be a steady rise in the number of cases in the coming future due to rapid industrialization and high population growth. The inhalation of these pollutants, irritants, noxious particles and harmful gases should cause deteriorating health conditions in the present patient population.

Inhaling the mentioned pollutants and gases and the complex interplay of genetic, environmental, and prevailing health condition factors contribute to the prognosis of the multifaceted disease of COPD. COPD is commonly characterized by parenchymal destruction, lung tissue remodelling, alveolar detachment, changes in pulmonary circulation, small airway disease and airflow obstruction. The degree of obstruction varies from person to person. The two most commonly existing conditions in patients that sums up this heterogenous disease are emphysema and chronic bronchitis. Emphysema is a condition that affects the air spaces distal to the terminal bronchiole and is caused by the permanent destruction of the lung's parenchyma. Chronic bronchitis is the inflammation of bronchioles which results in the hyper-accumulation of mucus in the tubes, causing difficulty in breathing even further. Due to chronic inflammation, the narrowing of small airways and destruction of lungs parenchyma results in loss of alveolar attachment, leading to decreased lung capacity of gaseous exchange. This limits the ability to expand of lungs. All these sum up only to one fact of lungs losing the forced expiration capacity causing FEV1 and FEV1/FVC ratio to drop and contributing to gas trapping.

The prognosis and pathogenesis of COPD are characterized by the generation of reactive oxygen species (ROS), which are responsible for causing mutations and inflammation. Even after ceasing of smoking habit, the progressive inflammation carries on. To neutralize ROS produced, the body's

first line of defence is antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase. Due to the alterations in the genotypes of these enzymes, whether in the coding or non-coding regions of the genes of these enzymes, the efficiency of the enzyme production or activity can be drastically reduced, causing severe damage to the body by un-neutralized ROS with increasing concentration, therefore, multiplying the probability of disease severity, pathogenesis and susceptibility.

Our case-control study comprised 200 clinically evaluated patients with different levels of disease severity and 200 moderately healthy controls. Our study aimed to evaluate the role of SOD1 and catalase polymorphism contributing to the risk of COPD prognosis. The mean age of the controls was 57.31 ± 7.59 and 58.54 ± 11.425 for cases. There was no significant difference in the age of cases and controls taken for our study. Cases mainly consisted of past or present smokers. The mean pack years of cases were 80.0 ± 40.76 while 17.26 ± 12.89 for controls having a significant difference in smoke intake between cases and controls. Faramawy et al. and Mehrotra et al. reported that the average pack-years of COPD cases were 35.3±7.0 and 20.8±4.7, respectively. This indicated that fewer pack-years of smoking are related to COPD susceptibility. There was a varied level of disease severity among the patient group. GOLD A comprised 29 (14%) patients with few symptoms and a low risk of exacerbations; GOLD B formed 146 (73%) COPD subjects with more symptoms and a low risk of exacerbations. GOLD E includes 22 (11%) cases with more symptoms and an increased risk of exacerbations. In our study, 98% of patients had breathlessness, 28.5% cough and 25.0% expectoration. In their study of COPD symptoms, Kessler et al. reported that breathlessness was the most common symptom in the patients. Of 2431 patients studied in their study, 72.5% had breathlessness, 60.1% cough (n=861) and 70.9% expectoration (n=1100).

The 50 bp Ins/Del polymorphism of *SOD1* affects the upstream enhancers of the gene, negatively affecting the gene transcription rate. Due to lower levels of *SOD1,* oxidative stress builds up, affecting the disease severity. Among the 200 assessed for both cases and controls, 68.5% had the wild types genotype (Ins/Ins), 28.5% had heterozygous polymorphic genotype (Ins/Del), and 6.5% had homozygous mutant genotype (Del/Del). While in controls, 74% had the wild types genotype (Ins/Ins), 25% had heterozygous polymorphic genotype (Ins/Del), and 1.0% had homozygous mutant genotype (Del/Del). This percentage of polymorphic variants was quite different compared

to controls, suggesting its contribution to disease prognosis. It was also concluded after the assessment that the mutant genotype (Del/Del) produced approximately five to six folds increase in the COPD susceptibility risk factors. As *SOD1* constitutes about 85% of the total SOD concentration, it is clear from the increased odds ratio that high Un-neutralised ROS concentration, thus suggesting it possesses as the risk factor in the disease prognosis. No or very few studies of 50 bps Ins/Del polymorphism have been conducted in COPD. Though Sarabandi et al. showed that *SOD1* 50 bps Ins/Del polymorphism is significantly associated with the prognosis of bladder cancer in the Iranian population. Nasab et al. showed a significant association for developing cardiovascular diseases due to 50 bps Ins/Del polymorphism.

The link associating *SOD1* polymorphism and the CAT score and mMRC scale was studied for the first time in our study. The CAT scores of Del/Del mutant genotypes were found to be highly associated with COPD susceptibility risk possessing four to twenty folds risk factors compared to the ordinary person. COPD severity was also significantly associated with SOD1 polymorphism, as a positive association was found between the GOLD group and polymorphism. Disease severity was found to be affected by polymorphism in moderately and highly severe cases.

Catalase's 262 C>T polymorphism occurs in the 5' untranslated region, at -262 position C to T. This polymorphism affects the transcriptional factor binding in the promoter region and splicing regulation. Compared to C, the T allele has been shown to have lesser enzyme activity. Due to lower levels of *catalase* activity, oxidative stress builds up, affecting the disease severity. Few studies have also shown that the polymorphic variant of catalase is not transported into peroxisomes. Among the 200 assessed for both cases and controls, 55.5% had the wild types genotype (C/C), 34.5% had heterozygous polymorphic genotype (C/T), and 10.0% had homozygous mutant genotype (T/T). While in controls, 59% had the wild types genotype (C/C), 38.5% had heterozygous polymorphic genotype (C/T), and 2.5% had homozygous mutant genotype (T/T). The homozygous recessive percentage of polymorphic variants was found to be quite different compared to controls, suggesting its contribution to disease prognosis. It was also concluded after an assessment that the mutant genotype (T/T) produced approximately a four-fold increase in the COPD susceptibility risk factors.

As catalase is one of the main enzymes that neutralize H_2O_2, a decrease in its activity indeed affects the ROS balance of the body. The H_2O_2 concentration increases causing tissue and DNA damage. Very few studies have been conducted to study the effect of 262 C>T polymorphism of catalase in COPD patients. Mak et al. stated in their study that 262 C>T polymorphism of catalase did not show any significant association in the pathogenesis of the disease in the Chinese population. Although, our study produced different results suggesting a significant association with COPD susceptibility.

The link associating *Catalase* polymorphism and the CAT score and mMRC scale was studied for the first time in our study. The CAT scores of the T/T mutant genotype were found to be highly associated with COPD susceptibility risk possessing five to seven folds risk factors when compared to an average person. Two-fold disease severity was observed when less severe mMRC scores were compared to more severe scores, suggesting a contribution to the disease prognosis. COPD severity was also significantly associated with *Catalase* polymorphism, as a positive association was found between the GOLD group and polymorphism. Disease severity was affected by the polymorphism in moderately and highly severe cases.

Gene combinational studies of *SOD1* and Catalase further confirmed that mutant polymorphic variants significantly increase the risk of COPD prognosis.

With rising COPD cases and mortality, and trends showing more in the coming future, a much greater focus is required in understanding the disease prognosis and treatment. Due to no permanent cure for treating COPD-related pulmonary damage, preventive measures remain the only possible way out. Genetic markers can help detect diseases early on, treating them more effectively. It is pretty lamentable to say that no or very few studies have been attempted in India to identify genetic markers in the prognosis of COPD in the Indian population. Our study results show that 50 bp Ins/Del polymorphism of *SOD1* and 262 C>T polymorphism of *Catalase* can be used as biomarkers. They contribute significantly to the disease prognosis. Although, a more extensive population study shall be necessary to assess the relationship between COPD progression and pathogenesis.

CHAPTER 7

CONCLUSION

Our case-control study concerns the patients diagnosed in Government Medical College, Patiala. The following points are conspicuous from the present study:

- 50 bp Del/Del polymorphism in *SOD1* was significantly associated with the prognosis and pathogenies, contributing to the risk associated with COPD susceptibility.
- The 262 C>T polymorphism of *Catalase* was also significantly associated with the prognosis and pathogenies, contributing to the risk associated with COPD susceptibility.
- In the case of the combinational effect of both polymorphisms, the risk of COPD susceptibility significantly increased with the genotypic alterations in the genes.

In conclusion, this study indicated that polymorphism in two primary antioxidant enzyme genes significantly modulated the risk of COPD prognosis in the North Indian population. These polymorphisms can be used as biomarkers to develop future diagnosis and therapeutic tools.

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