Impact of *GSTM1*, *GSTT1*, and *IL-4 R(α)* genetic polymorphisms toward susceptibility for Chronic Obstructive Pulmonary Disease (COPD)

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BY

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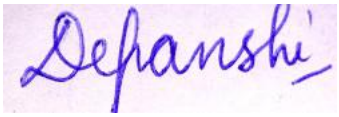
June 2022

DECLARATION

I hereby declare that the research work presented in the thesis entitled, "**Impact of *GSTM1, GSTT1,* and *IL-4 R(α)* genetic polymorphisms toward susceptibility for Chronic Obstructive Pulmonary Disease (COPD)**" has been carried out by me under the supervision and guidance of **Dr. Siddharth Sharma**, Associate Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala.

Further, I declare that no part of this thesis has been submitted for a degree or any other qualification of any other university or examining body in India/ elsewhere.



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CERTIFICATE

This is to certify that dissertation entitled, "**Impact of *GSTM1*, *GSTT1*, and *IL-4 R(α)* genetic polymorphisms toward susceptibility for Chronic Obstructive Pulmonary Disease (COPD)**" submitted by Ms. Depanshi Pandit in partial fulfilment of the requirements for the award of Masters of Technology in Biotechnology at Thapar Institute of Engineering and Technology, Patiala, is an authentic work carried out by her under my supervision and guidance.

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

Dr. Siddharth Sharma

Associate Professor
Department of Biotechnology

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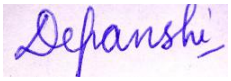

Depanshi Pandit

CONTENTS

Abbreviations

COPD	Chronic Obstructive Pulmonary Disease
GSTM1	Glutathione S Transferase mu (μ) 1
GSTT1	Glutathione S Transferase theta (Θ) 1
IL-4R(α)	Interleukin 4 Receptor Alpha
mMRC	Modified Medical Research Council
CAT	COPD Assessment Test
GOLD	Global Initiative for Chronic Obstructive Lung Disease
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
BSA	Bovine Serum Albumin
SDS	Sodium Dodecyl Sulfate
TBE	Tris Borate EDTA
TE	Tris EDTA
dNTPs	Deoxynucleotide triphosphates
OR-	Odds Ratio
AOR	Adjusted Odds ratio
CI	Confidence Interval

ABSTRACT

The progressive inflammatory lung disease known as chronic obstructive pulmonary disorder (COPD) is characterised by persistent respiratory symptoms and restricted airflow. Due to the intricate interactions between genetic and environmental factors, the pathophysiology of COPD is complex and heterogeneous. Numerous studies have looked into the relationship between the polymorphisms in the genes *GSTM1*, *GSTT1*, and *IL-4R(α)* and the risk of COPD in different ethnic groups and subgroups. However, the information we have so far is inconsistent. We conducted the study to look into the potential impact of the polymorphisms in *GSTM1*, *GSTT1*, and *IL-4R(α)* on the likelihood of developing COPD, either individually or in combination. For *GSTM1* and *GSTT1* polymorphisms, a total of 400 subjects (200 cases and 200 controls) were examined, and a total of 405 subjects (202 cases and 203 controls) were examined for *IL-4R(α)* polymorphism. Multiplex PCR was used to genotype the GSTM1 and *GSTT1* genes, and PCR-RFLP was used to genotype the *IL-4R(α)* gene. Compared to controls, COPD cases (34.5%) had a higher rate of *GSTT1* gene deletion (20.5%). The *GSTT1(-)* null genotype was found to have a significant statistical association with COPD risk (OR = 2.04, p = **0.0019**, 95 percent CI = 1.30-3.20). A strong correlation between the null genotype of *GSTT1(-)* and COPD risk was discovered after adjusting for covariates like age, gender, and smoking status (AOR =2.90, p=**0.003**, 95 percent CI=1.43-5.87). Additionally, a significant relationship between clinical parameters for COPD risk and *GSTT1(-)* genotype was observed. Another major observation was that females with *GSTT1(-)* null genotype were more vulnerable to COPD than males with the same gene deletion. However, no such significant association was observed in *GSTM1* and *IL-4R(α)* polymorphisms towards the COPD susceptibility. In conclusion, *GSTT1* polymorphism might play a vital role as biomarker for clinical prediction in COPD.

INTRODUCTION

Chapter 1

Introduction

In humans and many other vertebrates, the lungs are the principal organs of the respiratory system. They are a pair of spongy, pinkish-grey organs in the thoracic cavity of the chest. Humans usually have two lungs on each side, a right lung and a left lung. The left lung is relatively small than the right lung, which shares space in the chest with the heart. The lungs constitute the lower respiratory system, which begins with the trachea and branches into the bronchi and bronchioles. Bronchioles divide further into alveolar ducts, which give rise to alveolar sacs, which contain the alveoli, where gaseous exchange occurs. Several respiratory diseases, such as pneumonia, lung cancer, asthma, and others, can affect lung tissue and its parts, reducing overall function. However, the deadliest of all pulmonary disorders is Chronic obstructive pulmonary disease (COPD).

The third leading cause of fatality worldwide is COPD (World Health Statistics 2021) and the second leading cause of mortality and disability-adjusted life years (DALYs) in India, according to the global burden of disease (GBD) survey (Salvi *et al*., 2021). Over 80% of these fatalities are credited to low- and middle-income nations (World Health Statistics 2021). It is a significant public health concern and the leading cause of morbidity and mortality globally, resulting in a considerable increase in socio-economic burden (Lozano *et al*., 2012; Vos *et al*., 2012). COPD is a multifaceted inflammatory lung disease that causes gradual airflow obstruction Emphysema and chronic bronchitis are the most severe medical condition that induces COPD (McDonough *et al*., 2011). It is characterized by recurrent dyspnea and cough, and other symptoms encompass chest tightness, a change in appetite, weight loss, tiredness, and frequent pulmonary infection. COPD occurs as a consequence of a combination of genetic and environmental elements. In addition to environmental and genetic

risk factors, tobacco smoke exposure or cigarette smoking is the most crucial component associated with COPD development (Lokke*et al*.,2006). Even though the pathogenesis of COPD is unclear, studies have focused on four elements: (a) lung inflammation, (b) oxidation and antioxidation imbalances, (c) lung protease and anti-protease system imbalances, and (d) autonomic nervous system dysfunction, such as abnormal cholinergic receptor distribution (Cao *et al*., 2017). Furthermore, due to a reduced diet, there is a deficit of antioxidant micronutrients such as vitamin C, E, and so on in heavy smokers (Eiserich*et al*., 1995; Kallner *et al*., 1981; Lykkesfeldt *et al*., 2000). The only anti-protease associated with COPD is α1 -antitrypsin (1-AT) (Eiserich *et al*., 1995). If patients with the α1-AT gene deficiency smoke, they are more prone to develop COPD at a younger age (Kallner*et al*., 1981). According to the studies, smokers have a lower intake of antioxidants and higher oxidants due to their exposure to hazardous products, contributing to an elevated COPD risk (Traber *et al*., 2000). Nevertheless, only 25% of long-term smokers developed COPD (Lokke*et al*.,2006). The best possible pharmacologic therapy delivered at the appropriate point of care can alleviate COPD symptoms. It can reduce the intensity and frequency of COPD exacerbations and improve exercise capacity and overall wellbeing. COPD is frequently misdiagnosed, especially in the early stages, and no efficacious COPD medications are currently available to revert damaged lung functioning (Vestbo *et al*.,2013). It has been indicted by different studies that COPD development is due to the genetic variations in the enzymes that detoxify cigarette smoke products. Microsomal epoxide hydrolase (mEPHX), cytochrome p450 1A1, and glutathione S-transferase (GST) are among the oxidation-inhibiting enzymes (Yim *et al*.,2000). When genetic variation reduces the production or activity of oxidation inhibiting enzymes, the dynamic balance of oxidation/antioxidation is disrupted, resulting in oxidative damage (Zhang *et al*., 2015). GST is one of the most investigated genes in different human populations, and it is associated with the metabolism of

endogenous and environmental xenobiotics and the susceptibility to COPD (16). GST is a phase II biotransformation enzyme that detoxicates. It can be cytoplasmic, membrane-bound, mitochondrial, or leukotriene C4 synthase. There are eight mammalian categories of these enzymes denoted by Greek letters, namely α, μ, π, σ, θ, κ, ζ, and Ω (Nebert *et al*., 2004). The conjugation of reduced glutathione and GSTs removes the hazardous electrophilic compounds formed by oxidative stress (Rezaei *et al*., 2013). They are needed for organisms to respond to physiochemical stimulation in the environment and protect cells, proteins, and nucleic acids from free radical damage. Data has suggested that GST enzymes bind to toxic substances found in cigarettes, which act as substrates for biotransformation, thus guarding cells against carcinogenic and cytotoxic factors (Tang *et al*., 2013). Gene deletion is the most frequent cause of *GSTM1* and *GSTT1* null alleles (Lakhdar *et al*., 2010). Homozygous individuals invalid for *GSTM1* and *GSTT1* will lack the correlating enzyme function. Researchers have revealed that *GSTM1* and *GSTT1* gene deletions have been connected to the onset and progression of COPD (Lakhdar *et al*., 2011; Shukla *et al*., 2013). The *GSTM1* and *GSTT1* genes are expressed in the respiratory tract. *GSTM1* and *GSTT1* are found on 1p13.3 and 22q11.23, respectively. *GSTT1* is in charge of detoxifying conjugated lipid peroxide and halogenated compounds, whereas *GSTM1* is detoxifying benzopyrene diol epoxide (Cao *et al*., 2017). People with *GSTM1* null or *GSTT1* null genetic variants have reduced or no GST enzyme activity, making them more vulnerable to COPD-causing environmental factors. *GSTM1* and *GSTT1* gene deletions have been related to diabetes, rheumatoid arthritis, systemic sclerosis, bronchial asthma, Parkinson's disease, and various cancers (Mastana *et al*., 2013; Ji *et al*., 2013; Tew *et al*., 2001; Birbian *et al*; 2012; Pinhel *et al*., 2013; Chirilă *et al*., 2014; Krüger *et al*., 2015; Safarinejad *et al*., 2013; Sharma *et al*., 2015).

Inflammation is an immune system’s protective response against various xenobiotic and endobiotic harmful compounds. But in case of chronic inflammation in COPD, the

inflammation process continues extensively and aggravate the physiology of lungs. Accumulation of various inflammatory cells, such as neutrophils, mast cells, lymphocytes, dendritic cells, macrophages, CD8+ T-cells, B cells are implicated in the progressive destruction of the lungs in COPD (Chung *et al*., 2008). Cytokines play a vital role in orchestrating the chronic inflammation linked to COPD (Barnes *et al*., 2008). Cytokine *IL-4*located on 5q31.1 chromosome having five exons, plays a key mediator role in intercellular signalling network. IL-4 promote eosinophilic inflammation through the release of chemoattractant such as CC-chemokine ligand 26 (CCL26) from airway epithelial cells. They affect mucosal connective tissue fibroblasts and stimulate mucus hypersecretion, airway fibrosis and remodelling in COPD.

The susceptibility to COPD is polygenic. Several investigations have demonstrated polymorphisms in*GSTM1*, *GSTT1* and *IL4R(α)* in different ethnicities of COPD people. The relationship of *GSTM1*, *GSTT1*and *IL4R(α)* polymorphisms with COPD progression, on the other hand, was inconclusive across nationalities. Our study aimed to examine the role of *GSTT1*, *GSTM1*and *IL4R(α)*polymorphisms as a genetic marker for COPD risk in the North Indian population. Besides that, this is the first study to find a link between *GST* and *IL4R(α)* polymorphisms and various clinical parameters for COPD risk in the North Indian populace.

REVIEW OF LITERATURE

Chapter 2

Review of Literature

2.1 Lungs

The respiratory system's most crucial component is the lungs. The lungs are located in the thoracic chamber, an air-tight chamber anatomically. The thoracic section is formed dorsally by the vertebral column, ventral by the sternum, laterally by the ribs, and lower side by the dome-shaped diaphragm. Due to the anatomical arrangement of lungs in the thorax, any change in thoracic cavity volume is reflected in the lung (pulmonary) cavity.

The lung has three borders, three surfaces, and an apex anatomically. Above the first rib is the apex. The anterior, posterior, and inferior borders are the three borders. The pleural reflection corresponds to the anterior border of the lung, establishing a cardiac notch in the left lung. A cardiac gap is a concavity in the lung that constitutes the heart. The thin inferior border disaffiliates the lung base to the coastal surface. The thick posterior frame runs from the C7 to T10 vertebras and from the lung's apex to the inferior border. The costal, medial, and diaphragmatic surfaces are the three surfaces of the lung. The costal pleura encompasses the costal surface, which runs along the sternum and ribs. The costal surface joins the anterior and posterior borders, it also connects the medial and diaphragmatic surfaces, and at the inferior wall, it combines the medial and diaphragmatic surfaces. The medial surface is divided into anterior and posterior surfaces. It is connected to the sternum anteriorly and the vertebra posteriorly. Because of the liver, the diaphragmatic surface (base) is concave and rests on the diaphragm's dome; the left crown is shorter than the right dome.

The anatomy of the right and left lungs are similar but asymmetrical. The right upper lobe (RUL), right middle lobe (RML), and right lower lobe (RLL) are the three lobes that constitute the right lung. The left lung is differentiated into the LUL (left upper lobe) and the

LUL (left lower lobe). The lobes are further divided into segments corresponding to different segmental bronchi. The third-order branches of the second-order branches (lobar bronchi) that come off the main bronchus are known as segmental bronchi. The alveoli, which are present in the lungs, transport oxygen from the air to the blood. The alveoli are single-cell membranes that allow gaseous exchange with the pulmonary system. The diaphragm and intercostal muscles are two muscles that aid in inspiration and expiration (Chaudhry *et al*.,2021).

2.2 Chronic obstructive pulmonary disease

For over 200 years, Chronic obstructive pulmonary disorder (COPD) has been acknowledged. It is the most common lung disorder with the highest number of cases of morbidity and fatality across the world. It is characterized by persistent airflow limitation in the lungs due to alveolar attachment loss, inflammation, mucus obstruction in the luminal space, destruction of the lung parenchyma, and loss of lung elasticity. Breathlessness and cough are the most significant symptom among COPD patients. COPD was first diagnosed in the 19th century based on the symptoms and signs of a hyperinflated chest and decreased expiratory breath sounds (CPW Warren *et al*.,2009).

2.2.1Epidemiology of COPD

Prevalence and Mortality of COPD worldwide

The third common cause of mortality worldwide is COPD, with 3.23 million deaths in 2019 (WHO,2021). In 2016, 251 million cases of COPD were reported globally, and it is approximated that COPD ends up killing 3.15 million people each year (WHO,2017). In 2015, COPD impacted 299 million people, a 44% increase from 174 million in 1990 (Collaborators *et al*.,2017). According to a 2015 literature review of demographical studies from 52 countries, the Americas had the highest COPD prevalence (15% in 2010), while

Southeast Asia had the lowest (10%). The report suggested a global prevalence of 12%, roughly equivalent to 384 million cases in 2010, considerably higher than the GBD report's estimate. The eastern Mediterranean region experienced a tremendous percentage increase in COPD cases (119%) between 1990 and 2010, accompanied by the African region (102%), and the European region experienced the slightest increase (23%) (Adeloye *et al*.,2015). COPD accounted for nearly 64 million DALYs in 2015, ranking eighth among global disease burden causes. Papua New Guinea, India, Lesotho, and Nepal had the highest age-standardized DALY rates (>2000 per 100,000 people) due to COPD (Collaborators *et al*.,2017).

COPD prevalence in India

COPD is responsible for more than 9.5% of all deaths in India (Salvi *et al*., 2018). COPD is responsible for more than half of India's chronic respiratory disease burden and 70% of disability-adjusted life (Salvi *et al*.,2009). A study reported that India's COPD burden would likely range from 25.1 million to 49.2 million (Daniel *et al*.,2021). Noncommunicable diseases accounted for three out of the top five leading causes of death in 2016, with COPD ranking second (ICMR,2017). In various demographical studies conducted all over India, the prevalence ranged from 2% to 22% in men and 1.2% to 19% in women (Jindal *et al*.,2001).

Numerous reports have identified a variety of COPD prevalence in different states. It has risen to the fourth major cause of death in Empowered Action Group (EAG) states such as Jharkhand, Bihar, Madhya Pradesh, Odisha, Chhattisgarh, Rajasthan, Uttarakhand, and Uttar Pradesh. COPD was ranked seventh among North-Eastern regions, including Arunachal Pradesh, Assam, Meghalaya, Mizoram, Nagaland, Tripura, Manipur, and Sikkim. COPD was ranked fourth among the causes of death in the remaining Indian states. The highest number

of mortalities from COPD was nine times the lowest among all states in this highly variable range of disease burden (ICMR,2017).

Furthermore, COPD has been identified as one of the top eight leading causes of disability in all states. COPD-related DALYs increased 36.3% between 1990 and 2016, making it the second-largest cause of DALYs in India, trailing diarrheal disease, lower respiratory tract infections, stroke, and iron deficiency anaemia. Within the same time, the rate of incidence of COPD increased by 29.2%, posing a grave danger to human health. (ICMR,2017)

Economic Burden of COPD worldwide

In countries such as the United States, where COPD is the third major cause of death, the healthcare burden of COPD could be as high as $50 billion (Banerjee et al.,2014). The disadvantage is exceptionally high in low and middle-income nations due to its relationship with smoking and environmental pollution. According to estimates, these countries account for more than 90% of COPD-related mortalities, and India is no exception to such a massive public health issue (WHO,2017). COPD and its related comorbidities necessitate institutional resources and frequent hospitalization, which can be expensive for individuals and the healthcare system. Compared to younger patients, the elderly over 65 have a fourfold increase in hospitalization. In normal 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, adding a significant burden to the person and population level (CDC,2014). Insufficient data on the expenditure on COPD in India necessitates additional research to establish cost-effective solutions. In India's mixed health market, the financial and economic burden of treating illness is out of reach for most people, with out-of-pocket spending accounting for 62.41% of the overall health expenses (World

Bank,2014). Furthermore, India's social structure does not have appropriate safeguards for older people, leading to lower treatment adherence. (Hossain *et al*.,2018)

Rural-Urban Disparity of COPD

In 2010, rural counties accounted for 65% of all US counties, comprising 17% of the populace (Meit *et al.*,2014). Even after their lower population density, rural populations have worse health status than the urban population, increasing incidence, hospitalization, and the number of deaths. As per a 2015 cross-sectional assessment of Behavioural Risk Factor Surveillance System (BRFSS) records, people in rural areas had the highest Medicare hospitalization and mortality from COPD (Croft *et al*.,2018). Within a 16-kilometre (10-mile) radius, approximately 30% of rural residents have access to pulmonologist assistance than 90% of urban residents (Croft *et al*.,2016). Due to a pulmonologist shortfall, COPD management in rural locations is made available by primary care providers (PCPs), who frequently lack access to effective spirometry (Han *et al*.,2016). NHANES data from 2007 to 2012 was examined, and it was found that living in rural areas was linked with a 106% higher risk of COPD than living in the city. Regardless of the absolute incidence rates published, these studies demonstrate that rural populations are at an elevated danger of COPD (Raju *et al*.,2020).

2.2.2 Causes of COPD

The two most common ailments that induce COPD are emphysema and chronic bronchitis illustrated in **figure 2.1**. These two problems are frequently present simultaneously and vary substantially among COPD patients.

Emphysema is a lung disorder. This lung disease damages the alveoli's fragile walls and elastic fibres. As a result, there are fewer larger air spaces than the usual small ones. The

lungs' overall surface area is reduced due to fewer bigger air sacs. It means less oxygen may be transported into the bloodstream from the air we breathe.

Chronic bronchitis is a lung ailment marked by inflammation and narrowing bronchial tubes. The condition also leads to more mucus production, further plugging the narrower lines and causing a chronic cough to clear airways.

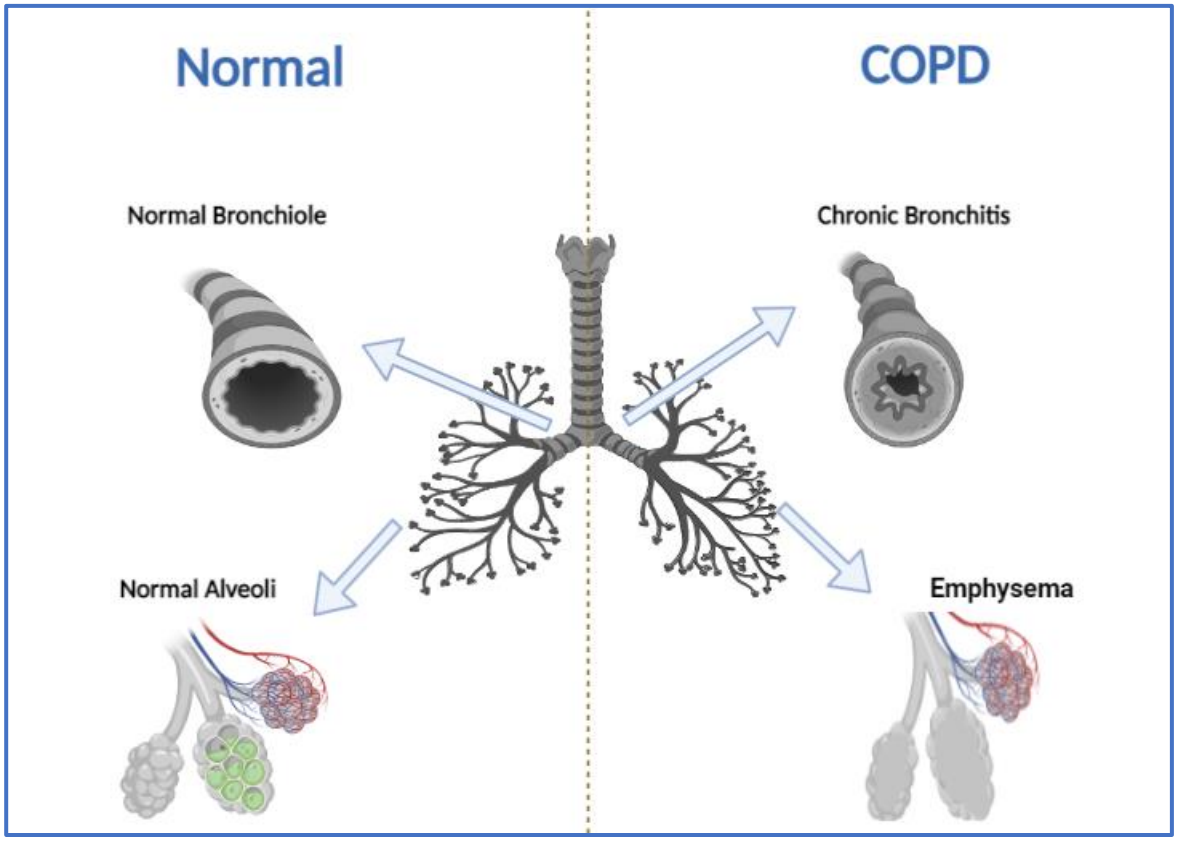

Figure 2.1: A diagrammatic representation illustrating healthy lung versus COPD lung

2.2.3 Risk factors of COPD

***Tobacco smoke exposure*:** The essential factor for COPD risk is cigarette smoking. Smokers of pipes, cigars, and marijuana are all in danger. Smoking is the leading cause of COPD in the United States and other high-income countries. However, air pollution is the second most dangerous factor globally, leading to COPD (Ruvana *et al*.,2020). In 2017, there were 1.23 million COPD-related fatalities and 28.28 million COPD-related DALYs due to active

smoking (Huang *et al*.,2019). Even though tobacco smoking is significantly linked with COPD risk, the uneven development of chronic airway obstruction among smokers infers that other factors influence disease expression (Barnes *et al*., 2007). Previous research estimated that smoking accounts for 15% of the variability in lung function, with genetic factors accounting for the remaining 40% (Coultas*et al*., 1991).

***Second-hand smoke exposure*:** Second-hand smoking, also called passive smoking, is the inhalation of tobacco smoke, or environmental tobacco smoke, by persons other than the intended "active" smoker. Second-hand smoke exposure was projected to cause approximately 600,000 all-cause deaths and nearly 11 million DALYs yearly in a 2011 survey (Oberg *et al.*,2011). Second-hand smoke exposure, especially during childhood, is a significant risk factor for COPD worldwide, including in the United States (Eisner *et al.*,2005).

***Air pollution*:** Complex compounds of air pollutants are generated by industries, automobiles, trucks, and households hazardous to human health. Among all air pollutants, delicate particulate matter with an aerodynamic diameter less than 2.5 mm (PM 2.5) significantly impacts pulmonary health. In 2015, PM 2.5 exposure resulted in 4.2 million deaths and 103.1 million DALYs, accounting for 8% of all deaths worldwide and 4% of worldwide DALYs. Most mortalities occurred in low- and middle-income nations, primarily in East and South Asia (Cohen *et al*.,2015). While levels of air pollution in developed countries have been improving due to the implementation of upper limits and better urban planning, air pollution in developing countries, particularly those experiencing rapid industrialization, has become a significant problem globally (Ruvana*et al*.,2020).

***Household air pollution*:** Nearly 3 billion individuals worldwide cook with solid fuel (e.g., wood, animal dung, crop residues, charcoal), and many more heat their dwellings with solid

fuel (Sood *et al*.,2018). These same biomass fuels lead to pollution of household air. When such solid fuel is combusted inefficiently, a complex mixture of carbon-based particles, inorganic particles, and irritant gases is produced indoors, comparable to tobacco smoke (Ruvana *et al*.,2020). Indian, Sub-Saharan African, Central American, and Chinese countries have the highest distributions of their populations exposed to indoor air pollutants (Sood *et al*.,2018). Even though the exposure burden is most significant in low-income nations, most houses in high-income countries, including the United States of America, depend on solid fuel to warm their residences (Sood *et al*.,2010). In 2017, indoor air pollution caused approximately 2 million deaths and 60 million DALYs worldwide, almost entirely in low- and middle-income countries, according to the Global Burden of Disease study; published WHO estimates are even more significant (Collaborators GBDRF,2017).

***Occupational exposures*:** According to a 2019 ATS statement, pooled projections of the population attributable fraction (PAF) are 14% for the work-related contribution to the COPD burden and 13% for chronic bronchitis. Furthermore, a higher occupational PAF for COPD among non-smokers (31%) implies that occupational exposures significantly contribute more to the burden of COPD in non-smokers. This finding indicates that as the general population's prevalence of cigarette smoking declines, other COPD-related determinants could become more prominent. Cigarette smoking and occupational exposure pose additive dangers for COPD, and it is evident that any of these exposures include a diverse set of hazardous pollutants (Blanc *et al*.,2019). COPD has been linked to occupational exposures to vapours, gases, dust, or fumes (VGDF) (Bang *et al*.,2013). The literature is well-documented on causal associations of COPD with silica, coal dust, construction dust, asbestos, cotton dust, and grain dust (Bang *et al*.,2015).

Nevertheless, numerous other at-risk occupations have gone unnoticed. A recent study revealed that employees in the information industry (including broadcasting,

telecommunications, publishing, and data processing workers) and office and administrative support occupations had the highest prevalence of COPD (including administrative and dental assistants, secretaries, and clerks). Employees in these industries are subjected to organic and inorganic dust, noxious gases, paper dust, isocyanates, photocopier fumes, chemicals, oil-based ink, toxic metals, paints, glues, and solvents, which are all known respiratory irritants related to COPD (Syamlal *et al*.,2019)

***Asthma*:** Asthma, a chronic inflammatory respiratory ailment, may increase one's chances of having COPD. Asthma and smoking both increase the likelihood of COPD. According to a study from the Tuscon Epidemiological Study of Airway Obstructive Disease, adults with asthma had a 12-fold higher risk of developing COPD over time than those without asthma, even after controlling for smoking (Silva *et al*.,2004). When the threat was adjusted, children with severe asthma were 32 times more likely to develop COPD. (Tai *et al*.,2014)

***Genetics*:** Throughout the most recent fifty years, there has been significant progress with minimal impact in understanding the genetics of COPD, which arose principally from an epidemiological question about why only a portion of smokers establish COPD while those with a comparable amount of smoking status do not. The only well-known genetic risk factor for COPD has been recognized: the SERPINA1 gene, which encodes for a serine protease inhibitor called alpha-1 antitrypsin (AAT). Antiproteolysis, inflammatory responses to cigarette smoke, toxic substance metabolism in cigarette smoke, and mucociliary clearance efficiency in the lung have all been linked to genes involved in COPD pathogenesis. Numerous studies have used association studies to look at candidate gene loci, comparing the allocation of variants in genes involved in COPD development in patients and controls. Polymorphisms in the genes for α1- AT, tumour necrosis factor (TNF)- α, microsomal epoxide hydrolase, α1-antichymotrypsin, vitamin D- binding protein, cytochrome P450 1A1, glutathione- S transferase, immunoglobulin- A and haem-oxygenase are all implicated in

COPD development in smokers (Yamada *et al*.,2000). Many relationships are ambiguous because they have not yet been reproduced (Sandford *et al*.,2001).

2.2.4Symptoms of COPD

- Shortness of breath, noisy breathing
- Frequent coughing, with or without sputum
- Wheezing
- Tightness in the chest
- Change in appetite, weight loss
- Tiredness
- Frequent infections of the lungs

2.2.5Comorbidities associated with COPD

COPD can cause many comorbidities such as lung cancer, respiratory infections, cardiovascular diseases, and neurological disorders. It has been discovered that most deaths in COPD patients are caused by complications rather than the disease itself (Berry *et al*.,2010).

***Lung Cancer*:** COPD is a common comorbid ailment in lung carcinoma, impacting 28.4%–39.8% of patients (Henschke *et al*.,2015). COPD could contribute to lung cancer by enhancing oxidative stress and the subsequent DNA damage, chronic exposure to pro-inflammatory cytokines, suppression of DNA repair mechanisms, and increased cellular proliferation (Durham *et al*.,2015). COPD is an independent predictor of lung cancer, primarily squamous cell carcinoma (Papi *et al*.,2004), and smokers with airway limitations are up to five times more likely to occur lung carcinoma than those with normal pulmonary function (Young *et al*., 2009; Durham *et al*., 2015).

***Respiratory infections*:** Either acute or chronic, respiratory infection plays a more prominent role in COPD progression and pathogenesis than already recognized. Because COPD is a

diverse condition, infection involvement may differ from patient to patient (Sethi *et al*.,2009). Individuals with chronic lung disorders are more prone to respiratory infections, and even some viral infections can promote disease pathogenesis. In the past, bacteria were expected to be the leading infectious aetiology of COPD exacerbations (Mallia *et al*.,2006). However, research suggests that viral upper respiratory tract infections (URIs) are the principal condition of COPD exacerbations (Proud *et al*.,2006). URIs are related to 40 % to 60 % of all COPD exacerbations, and viral infections have been indicated as crucial factors in COPD exacerbations (Tan *et al*.,2003). Indeed, respiratory viruses such as rhinovirus, respiratory syncytial virus (RSV), and influenza have also been linked to COPD exacerbations (Ko *et al*.,2007).

***Cardiovascular Disease*:** The most critical COPD-related comorbidities are cardiovascular diseases (CVDs) (Morgan *et al*.,2018). COPD patients are highly susceptible to cardiac disease, with a higher prevalence and incidence (age and gender-adjusted) when compared to patients without COPD. Indeed, fatalities from cardiac disease are higher in patients with moderate COPD than mortality from respiratory failure-related causes (Vestbo *et al*.,2016). Even in the general population, a high-risk period for cardiovascular events has been described immediately after acute respiratory infections (Clayton et al.,2008). The same is true after acute exacerbations in COPD patients, and a higher frequency of COPD exacerbations has been associated with a higher incidence of myocardial infarction (McAllister *et al*.,2012). Furthermore, cardiac biomarkers such as N-terminal proBNP (NT-proBNP), C-reactive protein (CRP), Vascular Endothelial Growth Factor (VEGF), and troponin are elevated in patients experiencing COPD exacerbations and are independent predictors for fatality (Chang *et al*.,2011). COPD prevalence among patients with heart failure (HF) ranges from 11% to 52 % in the United States and from 9% to 41% in Europe (Baum *et al*., 2016), whereas the occurrence of cardiac illness in COPD patients ranges from

14% to 33% (Mesquita *et al*.,2016). Since 2013, the susceptibility to and impact of heart illness in COPD patients have been acknowledged and incorporated in the guidelines (GOLD,2013).

***Neurological disorder*:** Neurological comorbidities, including anxiety, depression, and cognitive issues, are also prevalent in COPD patients and are linked to worsening symptoms and death rates (Pelgrim*et al*.,2019). Furthermore, transient ischemic attack, ischemic stroke, dementia, sleep disorders, and Parkinson's disease increase with age in COPD individuals (Puteikis *et al*.,2021).

2.2.6Diagnosisabout COPD

Spirometric interpretation of chronic obstructive pulmonary disease

GOLD (Global Initiative for Chronic Obstructive Lung Disease) and LLN (lower limit of normal) are two well-known definitions of COPD based on spirometric airway obstruction. The occurrence of two distinct meanings has hampered the interpretation of epidemiologic investigations. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have endorsed the GOLD definition (Ceilli *et al*.,2004), 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 (ERS) (Pauwels *et al*., 2001). Another ATS and ERS publication on spirometry interpretation methodologies recommend using the lower limit of normal (LLN) for the FEV1/FVC ratio to define airflow limitation (Pellegrino *et al*.,2005).

Spirometry is now the gold standard for diagnosing, staging and monitoring the progression of the disease. It is regarded as one of the most unbiased and repeatable measuring airway obstructions (GOLD,2007). Spirometry is strongly suggested for any patient with a prior history of exposure to COPD risk factors (e.g., smoking, occupational dust /chemicals), a history of severe lung ailment, or chronic symptoms of cough, sputum generation, or

shortness of breath (COPD,2007). Spirometry is used to evaluate a patient's FEV1, that is the volume of air forcefully exhaled in the first second of expiration after optimal inspiration, and FVC, which is the maximum amount of air exhaled until the lungs are emptied, which typically takes 3–15 seconds depending on disease severity. Spirometry must be conducted before and after patients receive an adequate dosage of short-acting inhaled bronchodilators to assess reversibility and rule out asthma. The patient's pulmonary function can be evaluated by calculating the ratio of the FEV1/ FVC. FEV1 and FVC readings and FEV1/ FVC ratios are lower in COPD patients than anticipated (reference) values depending on age, gender, height, and race (**Figure 2.2**). Airflow restriction has been diagnostically proven when the FEV1/FVC postbronchodilator value is <0.70. Advanced COPD is associated with FEV1 < 80% of predicted normal (GOLD,2007).

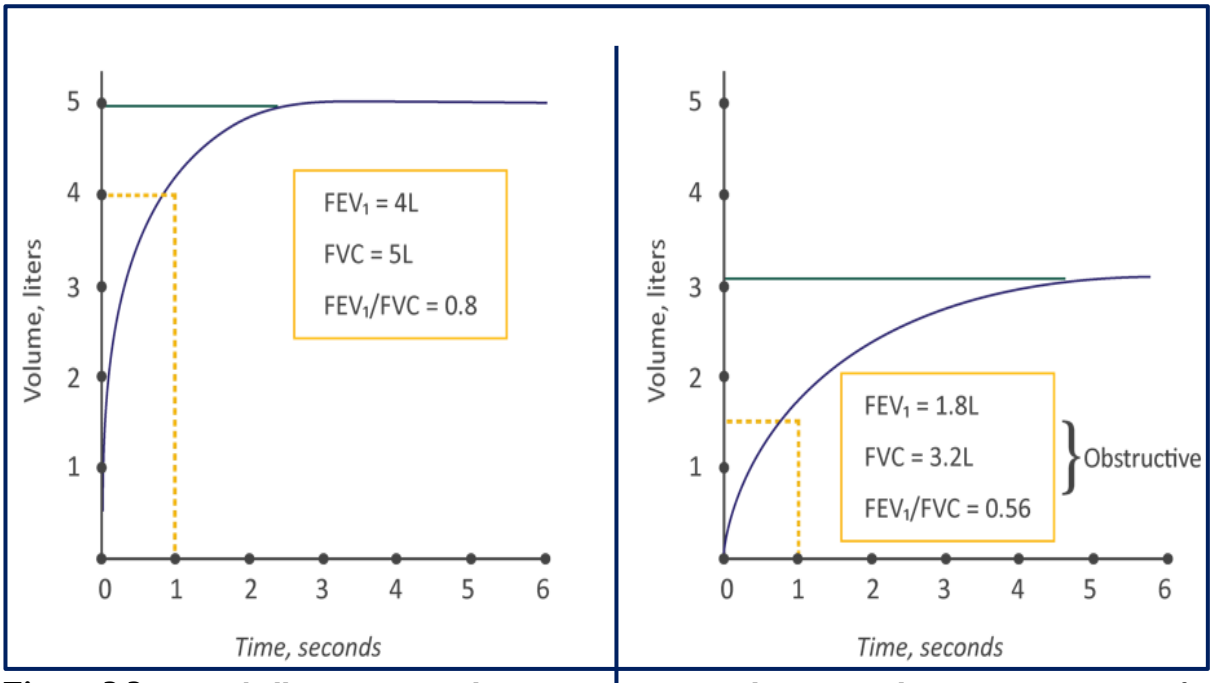

Figure 2.2: A graph illustrating typical spirometry tracing to depict a regular spirometry tracing of a patient with obstructive disease (right) where FEV1 and FVC are typically lower in COPD patients than in healthy individuals (left) (GOLD, 2021 report).

2.2.7 Treatment-related to COPD

Bronchodilators are the cornerstone of COPD maintenance therapy and are advised as first-line therapy for all GOLD groups (Vogelmeir *et al*.,2017). Patients in GOLD group A (low symptom burden and low exacerbation risk) are suggested to begin short- or long-acting bronchodilator treatment depending on its impact on breathlessness, while patients in GOLD groups B or C (high symptom burden and low exacerbation risk) obtain a long-acting bronchodilator as initial treatment. Combining LABA and long-acting muscarinic antagonist (LAMA), bronchodilators are prescribed for patients in GOLD groups B or C with persistent symptoms after bronchodilator monotherapy or as first-line therapy for patients in GOLD group D. (high symptom burden and high exacerbation risk). Remarkably, compared to LAMA or LABA monotherapy, combined treatment with a LAMA/ LABA improves FEV1 while reducing symptoms and exacerbations (Thomas *et al*.,2017). Bronchodilator therapy improves pulmonary emptying, reduces dyspnea and hyperinflation, and enhances the patient's ability to exercise; however, it cannot reverse the loss of lung function or improve the patient's condition (Fromer *et al*.,2008).

2.3 Detoxification system to eliminate xenobiotic compounds

Every day, the human body is surrounded by innumerable noxious xenobiotic constituents that must be eliminated from the body to avoid detrimental effects. Intricate enzymatic pathways remove toxic substances and defend the body inside the body. The primary critical players in exterminating such xenobiotic molecules are phase 1 and 2 enzymes and several other enzymes.

2.3.1*Phase 1 enzymes*

Phase 1 enzymes include the cytochrome p450 monooxygenase system, flavin-containing monooxygenases, NADPH cytochrome P450 reductase, epoxide hydrolases, and esterases. A new functional group is incorporated, or an existing active group is exposed with the help of

phase 1 enzymes to make the toxins electrically charged and water-soluble. It is accomplished through chemical reactions that include oxidation, hydration, hydrolysis, reduction, and dehalogenation (**Figure 2.3**).

2.3.2 *Phase 2 enzymes*

UDP-glucuronosyltransferases, N-acetyltransferases, sulfotransferases, methyltransferases, and glutathione S-transferases (most notably catechol O-methyl transferase and thiopurine S-methyl transferase) are Phase 2 enzymes. These enzymes participate in the conjugation reaction by adding or transferring polar compounds to the functional group, and the conjugates formed are more water-soluble and thus easier to eliminate from the body (**Figure 2.3**).

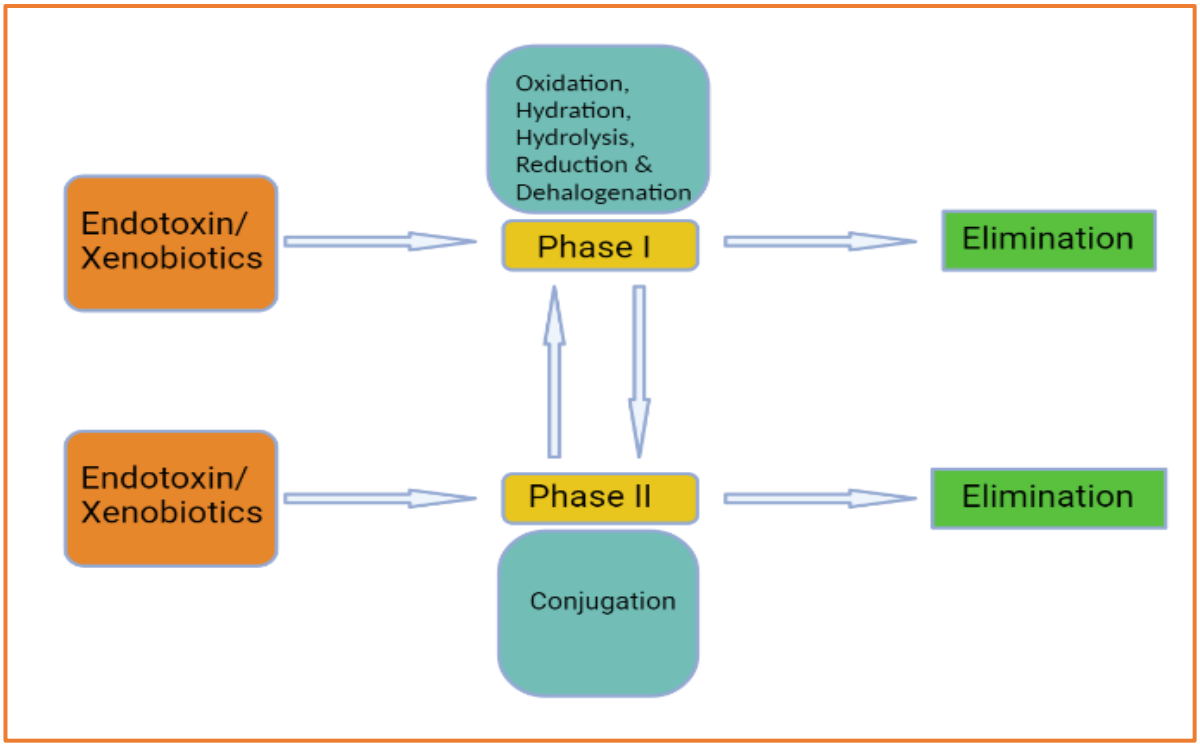

Figure 2.3: A schematic representation of the xenobiotic detoxification mechanism of phase 1 and phase 2 enzymes.

2.4 Glutathione S-Transferases

Glutathione S-Transferases (GST) (EC.2.5.18) is a member of the large family of phase II metabolic enzymes. It is best known for catalyzing the conjugation of reduced glutathione to electrophilic substances produced by xenobiotic or endobiotic substrates to inactivate and facilitate their removal from the body, thereby preventing oxidative damage biomolecules (Hayes *et al.*,2005). It was first discovered in animals in 1961 and was thought to be involved in drug detoxification (Booth et al.,1961). GST detoxifies carcinogens, environmental pollutants, chemotherapeutic drugs, and various xenobiotics (Dasari *et al.*,2018).

2.4.1 *GSTs families*

GSTs can be found in various subcellular compartments such as the cytosol, endoplasmic reticulum (ER), mitochondria, nucleus, and plasma membranes. Human GSTs are classified into three families: cytosolic, mitochondrial (kappa class), and microsomal GSTs, which are integral membrane proteins known as MAPEG (membrane-associated proteins participating in eicosanoid and glutathione metabolism). GSTs in the cytosol are also categorized based on sequence similarities and physical and structural characteristics. In humans, cytosolic GSTs are further classified into seven functional groups: Alpha(α), Mu(μ), Omega(Ω), Pi(π), Theta(Θ), Sigma(σ), and Zeta(ζ) as shown in below schematic representation, **figure 2.4**. Mitochondrial GSTs are soluble enzymes with structural similarities to cytosolic GSTs, whereas microsomal GSTs are evolutionarily distinct from the other classes of GSTs. (Vandewatering *et al.*,2021)

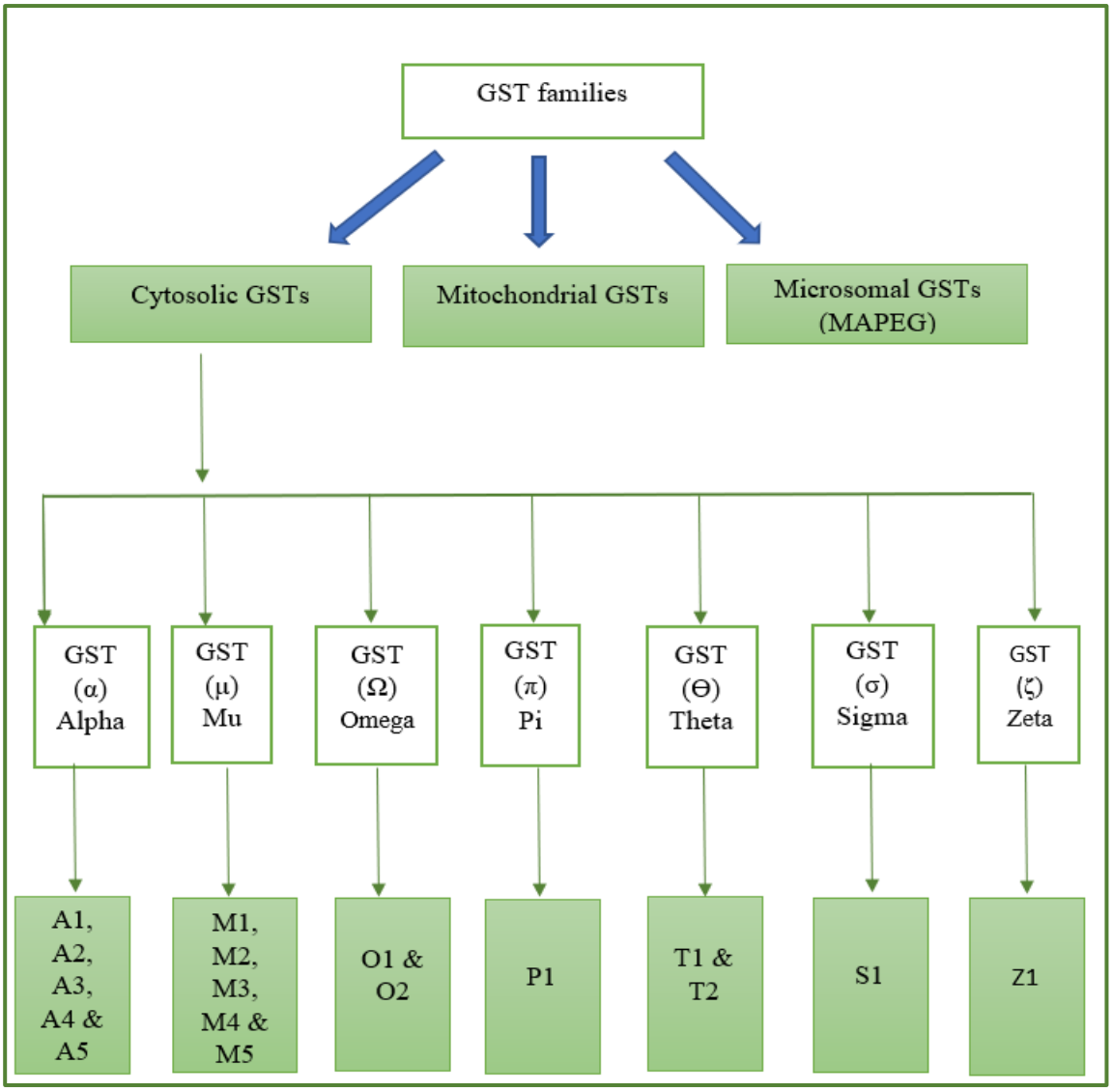

Figure 2.4: A flowchart showcasing the different types of human GST family members.

2.4.2 *Mechanism of the catalytic reaction of GSTs*

GSTs conjugate the reduced glutathione with numerous xenobiotics or endogenous compounds, mostly electrophilic molecules, to inactivate or excrete out from the body that may cause oxidative damage if present in the human body (**Figure 2.5**).

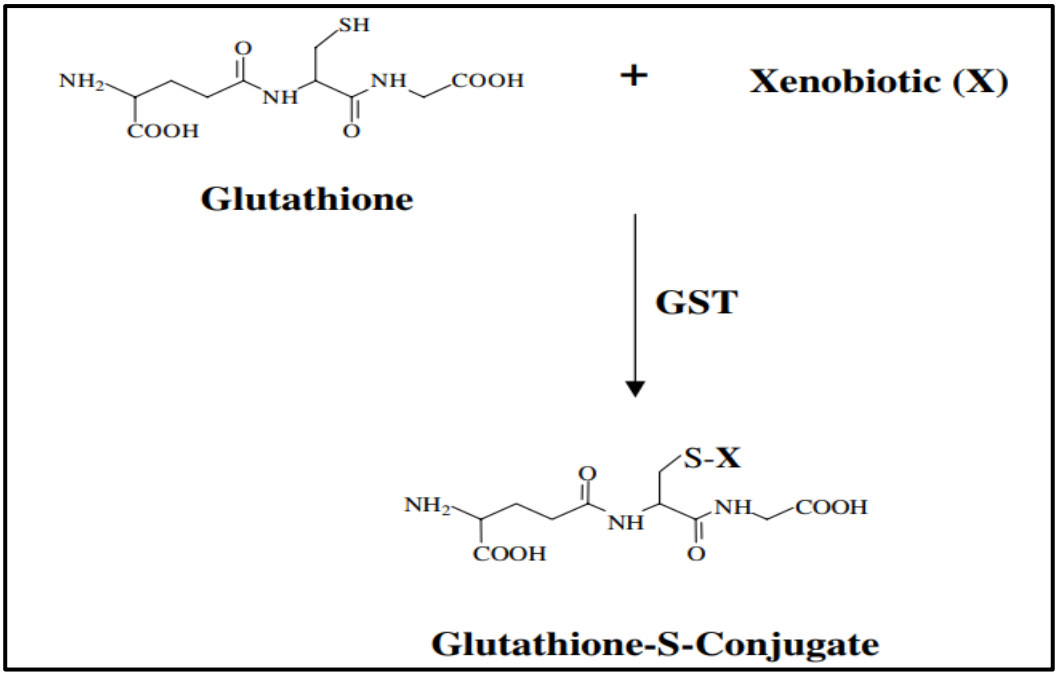

Figure 2.5: The catalytic reaction mechanism of GST on the xenobiotic substrate to form the glutathione-S conjugate (Townsend *et al*.,2003)

2.4.3*The primary role of GST*

- Detoxification
- Regulation of redox signalling
- Protein interaction
- Oxidant scavenging

GST classes	Location	Tissue Specific Expression	Function
GST A	Chromosome 6	Liver, kidneys, adrenal glands, intestine, testis, and other tissues, including the lung.	Metabolizes bilirubin and heme in the liver. *GSTA* enzymes also have GPX (Glutathione peroxidase) activity. *GSTA* enzymes are also known to have glutathione-dependent steroid isomerase activity (particularly *GSTA3*), as well as the movement toward polycyclic aromatic hydrocarbons, epoxides, and alkenyl products of lipid peroxidation (4-hydroxynonenal), particularly *GSTA4* (Coles *et al.*,2005).
GST M	Chromosome 1	Liver, lungs, muscle, kidney, & brain.	Detoxifies carcinogenic compounds such as aromatic amines, polycyclic aromatic hydrocarbons, and other organic compounds such as trans-stilbene oxide, styrene-7,8-oxide, and benzene (a)pyrene using 1-Chloro-2,4-dinitrobenzene (CDNB). *GSTM1* has also been shown to bind (non-substrate dependent) and inhibit the activity of apoptosis signal-regulating kinase 1 (ASK1), a MAPK kinase (MAPKKK) that activates c-Jun N-terminal kinase (JNK), a protein essential in stress responses and pro-apoptotic signalling, and p38 pathways, which regulate *GSTM1*catalyzes the forward PSSG reaction as well (Klaus *et al*.,2013).
GST O	Chromosome 10	Liver, notably in macrophages, glial, endocrine cells, and other tissues including kidney, lung, and gallbladder.	*GSTO* enzymes have low activity against common substrates like CDNB. They play roles in cellular redox homeostasis and modulate ryanodine receptors and calcium channels in the ER. It has been linked to anti-cancer drug resistance. It has also been proposed that *GSTO1* influences cell survival by activating survival and inhibiting the apoptotic signalling pathway. Furthermore, *GSTO1* has been shown to translocate to the nucleus in response to heat shock and other stress conditions, but its function is unknown. The *GSTO1* isoform has been identified as a di-arylsulfonylurea binding protein, and this interaction is thought to be responsible for inhibiting ATP-induced interleukin one beta (IL-1) posttranslational processing

			(Piaggi *et a*l.,2009).
GST P	Chromosome 11	Liver, heart, lung, and brain.	*GSTP* can bind to JNK via specific protein-protein interaction in a non-substrate-dependent fashion, inhibiting kinase activity and protecting cells from (H2O2-induced) cell death (Elsby *et al*.,2003). Another *GSTP* ligand binding partner is tumour necrosis factor receptor-associated factor 2 (TRAF2), a member of TNF-induced signalling that activates p38 and JNK and is inhibited by *GSTP* binding. Furthermore, *GSTP* is known to catalyze the forward PSSG reaction (Townsend *et al*.,2009).
GST S	Chromosome 4	Adipose, placenta, lung and foetal liver.	It contributes to the production of prostanoids in the immune system and mast cells. This enzyme's presence can determine the stage of differentiation of human megakaryocytes (Ricciotti *et al*.,2011).
GST T	Chromosome 22	Liver, kidney (renal proximal tubule cells), gastrointestinal tract, and lung	*GSTT1* plays a vital role in the phase II biotransformation of drugs and chemicals and the detoxification of substrate intermediates produced during oxidative stress, such as peroxidized lipids. GSTT also detoxifies smoke-derived small hydrocarbons such as ethylene oxide, epoxy butanes, and methyl bromide (Josephy *et al*.,2009).
GST Z	Chromosome 14	Endocrine tissues, liver, stomach, testis, pancreas, cytosol mitochondria.	*GSTZ* is involved in the catabolism of tyrosine as well as detoxification. It is also known as maleylacetoacetate isomerase (MAAI) because it catalyzes the cis-trans isomerization of maleylacetoacetate to fumarylacetoacetate. *GSTZ*, on the other hand, is the only enzyme in the GST family that contributes to oxidation by catalyzing processes in the intermediary metabolism, such as the conversion of dichloroacetic acid to glyoxylate in a reaction that requires but does not consume GSH (Anderson *et al*.,2004).

Table 2.1: Tabular chart representing various GST classes' function, location, and tissue-specific expression.

2.5 Role of GSTs in various diseases

GSTs have been found to play an essential role in a variety of diseases, including Alzheimer's, Parkinson's, stroke, epilepsy, gliomas, lung cancer, ovarian cancer, breast cancer, colorectal cancer, thyroid cancer, prostate cancer, cardiovascular diseases, rheumatoid arthritis, diabetes mellitus, asthma, allergies, and inflammatory conditions such as COPD, bronchitis, pneumoconiosis, and others (Kennedy *et al*.,2020).

2.6 Role of GST in COPD

Studies back up the common perception that GST deficiencies (particularly the *GSTM* and *GSTT* null polymorphisms) are linked to an increased risk of COPD development related to adverse perinatal impacts and impaired lung growth and development. Current findings have revealed that GST enzymes bind to many harmful, toxic compounds in cigarettes (such as oxidizing agents/free radicals) and serve as substrates for biotransformation metabolism, safeguarding cells from cytotoxic and cancer-causing variables. GSTs are, therefore, essential for the protection of the airway and alveolar epithelial cells and the prevention and treatment of COPD (Cao *et al*.,2017)

2.6.1 GST polymorphism and COPD

Even though smoking is the leading cause of COPD, hardly 10-20% of chronic heavy cigarette smokers develop symptomatic COPD, indicating the existence of genetic predisposition. This genetic predisposition to COPD may be due to variations in enzyme activities that detoxify cigarette smoke products, such as microsomal epoxide hydrolase (mEPHX) and glutathione-S transferase (GST). Since there is mounting evidence that many genes contribute to COPD development, multiple gene polymorphisms must be examined to determine genetic susceptibility to COPD (Yim et al.,2000). GST gene superfamily is the most studied of all the genes in the human population, and the null genotypes have been

linked to an increased risk of COPD. The study highlights the current research trends and developments in the association of GST polymorphism with COPD risk. The effect of deficiency of GST genes on COPD patients by different researchers is given below.

A study by Yim *et al*. depicted a combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S – transferase *M1* and *T1* for genetic susceptibility to COPD in the Korean population. The study included 159 participants, and their genotype was identified to associate with COPD development. However, they found no link between the genes mentioned above, individually or with COPD risk (Yim *et al*., 2000).

The study by He *et al*. analyzed polymorphism in several antioxidant genes and their association with the rate of lung function decline in smokers. The GSTM1 and GSTT1 deletions were detected using a multiplex polymerase chain reaction (PCR). A modified PCR-RFLP discovered the A313G (Ile105Val) genetic variation in the *GSTP1* gene. There was no statistically significant effect of the GST genotypes on lung function decline. When genotype combinations were examined, concurrent deletion of the *GSTT1* and *GSTM1* genes, along with the presence of the *GSTP1AA* genotype, was associated with an increased risk of accelerated decline in lung function in smokers. Furthermore, having homozygous *GSTP1 AA* and having a family history of COPD was linked to an increased risk of lung function decline (He *et al*., 2002).

A report by Gasper et al. determined the link of *CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1*, and *TP53* polymorphisms with COPD susceptibility and non-small cell lung cancer. The study included 262 Brazilians of European descent, with 97 patients with non-small-cell lung cancer (NSCLC), 75 individuals suffering from COPD, and 90 serving as controls. They discovered no significant relationship between the markers studied and NSCLC susceptibility. Even though the distribution of the *CYP1A1, GSTM1, GSTP1, GSTT1*, and

TP53 genotypes was similar in COPD patients and controls, the frequency of the *CYP2E1*1A/*5B* heterozygote was approximately six times higher in COPD patients than in controls (OR= 6.3; 95 % CI= 1.1-35.5, p = **0.04**). Participants with the *GSTT1* null phenotype and the *GSTP1 Ile/Val* genotype were about four times more likely to have COPD than those without these genotypes (OR= 4.0; 95% CI = 1.2–14.6, p = **0.02**), as were those with the *GSTT1* null phenotype and the CYP1A1*1A/*2A genotype (OR= 3.7; 95% CI = 1.1–14.6, p=**0.04**). These findings imply that the *CYP2E1* and *GSTT1 + GSTP* or *GSTT1 + CYP1A1* polymorphisms may predict COPD susceptibility, at least in the European ancestry population (Gasper *et al*., 2004).

An investigation was done by Cheng *et al*. on the Taiwanese population to determine genetic susceptibility to the development and severity of COPD. They concluded that COPD might result in variations in enzymes that detoxify cigarette smoke products, such as microsomal epoxide hydrolase (mEPHX) and glutathione S-transferase (GST). The polymerase chain reaction was used to determine the genotypes of 184 COPD patients and 212 matched controls, followed by RFLP analysis of the *mEPHX*, *GSTM1*, *GSTT1*, and *GSTP1* genes. All of the participants were smokers or ex-smokers. The proportion of *GSTM1*-null genotypes was significantly higher in COPD patients than in controls (61.4% vs 42.5%). For *mEPHX*, *GSTT1*, or *GSTP1*, there were no statistically significant differences in the distribution of polymorphic genotypes. During a combined analysis of genetic polymorphisms for *mEPHX*, *GSTM1*, and *GSTP1*, it was observed that there are strong predictors of COPD susceptibility (OR- 2.2, 95% CI- 1.3–3.5). In patients with severe COPD, the frequencies of homozygous mutant alleles of *mEPHX* exon three and the *GSTM1*-null genotype were significantly higher (Cheng *et al.*, 2004)

The research conducted by Yanchina *et al*. examined the incidence of allele variants of glutathione-S transferase *M1* xenobiotic detoxification gene and matrix metalloproteinase

nine genes in patients with COPD. Seventy-two COPD subjects were included in the investigation. *GSTM1* and *MMP9* allele variants were identified using PCR and subsequent restriction analysis. *GSTM1* (0/0) genotype was 2.5 times more prevalent in COPD patients than in control tobacco smokers. None of the participants had a homozygous *MMP9* (-1562T) mutation. The *MMP9* factor (CT) effect was relatively weak, and the OR did not reach statistical significance. However, it was potentiating for *GSTM1* 0/0 carriers, i.e., the risk of COPD in tobacco smokers with *GSTM1* (0/0)/*MMP9* (CT) genotype combination increases 7.7 times compared to carriers of two normal genotypes (Yanchina *et al.*, 2004).

The report by Calikoglu *et al*. determined the association between gene polymorphisms of glutathione S-transferases (GSTs) and COPD in the Turkish population. Real-time PCR evaluated the genotype of 149 patients and 150 healthy individuals for *GSTT1, GSTM1*, and *GSTP1* polymorphisms. The *GSTM1* and *GSTT1* genotypes did not differ between groups, but the *GSTP1 Ile/Ile* genotype was significantly higher in patients than in controls (61.1% vs 38 %). GSTM1, GSTT1 null and GSTP1 Val/Val were linked to a maximal increased (12-fold) COPD risk among the genotype combinations. As a result, they prophesied that investigating a single gene family would be insufficient to describe the etiopathogenesis of COPD (Calıkoglu *et al*., 2006).

A designed case-control study reported by Chan-Yeung *et al*. showed the role of polymorphisms of genes regulating glutathione S-transferase (GST) and its plasma GST activity in the pathogenesis of COPD in the Chinese population. A total of 163 cases and the same number of controls were used in the report. The genotypes and allele frequencies of *GSTM1, GSTT1,* and *GSTP1* in patients and controls were deduced, and the respective ORs. *GSTM1* and *GSTT1* null genotype frequencies were not different between COPD patients and healthy smokers. The frequencies of the three *GSTP1* genotypes and alleles were not different between COPD patients and healthy controls. Only 1.2 % of healthy controls and 4.9 % of

COPD patients had the *GSTP1 Val/Val* genotype. Combinations of different GST gene polymorphisms did not raise COPD risk. In COPD patients with varying levels of airway limitation, there were no significant differences in the frequencies of *GSTM1*, *GSTT1*, and *GSTP1* genotypes (FEV1% predicted) (Chan-Yeung *et al*., 2007)**.**

The research by Imboden *et al*. examined that the deficiency of GST enzymes led to the decline in lung function in the Spaldia populace. Regardless of smoking status, *GSTT1* homozygous gene deletion alone or in combination with *GSTM1* homozygous gene deletion was connected with an excess decline in FEV1 in men but not women. In men with *GSTT1* and concurrent *GSTM1* gene deletions, the additional mean annual decline in FEV1 was -8.3 ml/yr (95 % CI =12.6 to 3.9) compared to men without these gene deletions. Their findings indicated that genetic *GSTT1* deficiency is a common and significant predictor of expedited lung function decline in the general male populace (Imboden *et al*., 2007)

The study by Zidzik *et al*. determined the risk of chronic obstructive pulmonary disease (COPD) associated with polymorphisms in the glutathione S-transferase *GSTM1*, *GSTT1*, and microsomal epoxide hydrolase (*EPHX1*) genes in a cohort of Slovak population. The investigation included 377 Caucasians (217 patients with COPD and 160 control subjects). The *EPHX1 His113-His113* genotype at exon 3 was linked to an increased risk of COPD (OR= 2.32; 95% CI=1.20-4.69; p=**0.008**). However, this association was non-significant after adjusting for age, gender, and smoking status (OR= 1.79; 95% CI= 0.91-3.53; p=0.093). On the contrary, the *EPHX1* gene's exon four codon 139 polymorphism found no correlation with COPD risk. Besides that, no significant relationship was found between *GSTM1* or *GSTT1* genotypes and COPD risk. The polymorphisms in the *GSTM1, GSTT1,* and *EPHX1* genes were studied to evaluate their association with COPD risk. The combined *EPHX1 His113-His113/GSTM1* null genotype was linked to an increased risk of COPD in a combined analysis of gene polymorphisms for the *EPHX1, GSTM1,* and *GSTT1* genes (OR=

5.08; 95% CI= 1.70-20.43; p=**0.001**). After adjusting for age, gender, and smoking status variables, the association between the combined *EPHX1 His113-His113/GSTM1* null genotype and an increased risk of COPD remained significant (OR= 4.87; 95% CI= 1.57-15.13; p=**0.006**) (Zidzik *et al*.,2008).

The report by Dimov *et al*. investigated the relation of *GSTP1, GSTM1,* and *GSTT1* gene polymorphisms and susceptibility of COPD in Bulgarian citizens. A case-control study was conducted with 48 COPD subjects and 120 healthy subjects. The PCR RFLP method was used to determine genotyping. A null *GSTM1* (p=**0.003**, frequency=0.67 vs. 0.36) homozygous genotype was associated with a significant case-control difference but not with a *GSTT1* (p=0.364, frequency=0.13 vs 0.07) homozygous genotype. Patients with controls had a higher prevalence of at least one null genotype (p=0.060, frequency =0.72 vs 0.50). They discovered a 3.60-fold (95% CI=1.52-8.54) higher risk with the *GSTM1* null genotype and a 2.54-fold (95 % CI= 0.96-6.73) increased risk with carrying at least one GST null genotype. No relation was found between the frequency of *A313G (Ile105Val) GSTP1* alleles and genotypes and the likelihood of developing COPD (p=0.807 and p=0.958). They proposed that the inherited absence of GST-mu, but not GST-theta detoxifying enzyme, due to the prevalence of homozygous null genotypes may be related to COPD (Dimov*et al*., 2008).

This study by Faramawy *et al*. analyzed the link between the genetic polymorphism of glutathione S-transferase θ1 (*GSTT1*) and glutathione S-transferase μ1 (*GSTM1*) with COPD in smokers. A case-control study was performed on 34 COPD patients and 34 matched control participants. The prevalence of the null *GSTT1* genotype carriers in cases was 50%, compared to 44.1 % in the healthy controls. Pages of the null *GSTT1* genotype had a lower risk of developing COPD than carriers of the wild GSTT1 genotype (OR= 1.3; % CI= 0.5-3.3). In the case of *GSTM1*, 52.9 % carried the null GSTM1 genotype, compared to 26.5 % of controls. Carriers of an invalid*GSTM1* gene were more prone to develop COPD (OR=3.13;

95% CI=1.1-8.6). Furthermore, pages of the null *GSTT1* and *GSTM1* haplotypes had a greater chance of developing COPD (OR=3.6; 95% CI=1.1-11.6) (Faramawy *et al*., 2009)

The research investigated by Mehrotra *et al.* depicted the polymorphism of glutathione S-transferase *M1* and *T1* gene loci in COPD. Multiplex PCR–RFLP technique was used to assess genetic polymorphisms of the *GSTM1* and *GSTT1* genes in 50 COPD patients and 50 healthy controls to check whether these polymorphisms are linked to genetic susceptibility to COPD. When COPD patients were compared to controls (32.0%), the *GSTM1* homozygous null genotype frequency was 28.0 %. The difference was not statistically significant, implying that the *GSTM1* null genotypes were unrelated to COPD risk. *GSTT1* homozygous null genotypes were significantly higher in COPD cases than in controls (40 % versus 14.0 %), implying that the theta-glutathione S-transferases null genotype may be connected with COPD susceptibility. No significant differences were noted when GSTM1 and GSTT1 were compared based on disease severity and smoking. COPD was also found to develop at a younger age and with a shorter pack-year history in the Indian population (Mehrotra *et al*., 2010**).**

Report by Lakhdar *et al*. associated the *GSTM1* and *GSTT1* polymorphisms with COPD in 234 COPD patients and 182 healthy controls in the Tunisian population. Multiplex PCR was used for genotyping the samples. *GSTM1* null genotype was linked to an increased risk of COPD (OR = 1.58; 95% CI 1.06–2.35; p = **0.02**). The *GSTM1* null genotype was more common in the patient group (65.38 %) than in the controls (54.39 %). The association between *GSTM1* and COPD was insignificant after adjusting for gender, age, BMI, smoking status, and pack-year smoking using a logistic regression model (OR = 1.29; 95%CI = 0.41–3.86; p = 0.073). On comparison of the two groups revealed that the difference in *GSTT1* null genotype distribution between COPD cases (31.62%) and controls was not significant (p>0.05) (29.12%). The frequency of combined *GSTM1* and *GSTT1* null alleles in the patient

group was higher (22.64 % vs 18.68 %), but no significant relationship with pathogenesis was found (p> 0.05) (Lakhdar *et al*., 2010).

An investigation done by Thakur *et al*. examined the relationship of *GSTM1/T1* genes with COPD and prostate cancer in the north Indian population. The subjects evaluated included 200 COPD cases, 150 prostate cancer cases, 150 BPH cases, 200 age-matched controls for COPD, and 172 age-matched controls for prostate cancer. Multiplex PCR was used to identify the polymorphism. *GSTM1* null genotype was found in 38.5% of cases and 30.5% of controls. As a result, COPD patients had a higher proportion of null genotypes than controls. Compared to controls (11.5 %), the *GSTT1* null genotype increased (21.5%) in the COPD group. With an OR of 1.4 (OR= 1.43; 95% CI= 0.94–2.16), the *GSTM1* null genotype was associated with a slightly non-significant increase in COPD risk. *GSTT1* null genotype, on the other hand, was associated with a higher risk of COPD **(**OR= 2.11; 95% CI= 1.22–3.62; p= **0.007**). The *GSTM1* and *GSTT1* null genotypes were associated with a significantly increased (OR 3.73; 95 % CI 1.50–9.28; p= **0.004**) risk of COPD. When the *GSTT1* null genotype was combined with the *GSTM1* present genotype, the risk was reduced to two (OR= 1.83; 95% CI =0.94–3.56). There was a marginal risk of COPD with the *GSTM1* null genotype and *GSTT1* present genotype (OR= 1.33; 95 % CI =0.85–2.10). *GSTM1* and *GSTT1* null genotypes were found to be associated with a 2.5-fold (OR= 2.45; 95 %CI= 1.56–3.82; p= **0.00008**) and 2.4-fold (OR= 2.39; 95%CI= 1.36–4.20; p= **0.002**) increased risk of prostate cancer, respectively. Smoking was linked to a 2.2-fold increase in prostate cancer risk (OR= 2.23; 95% CI= 1.36–3.65, p = **0.001**) and a twofold increase in BPH (OR**=** 2.09; 95% CI= 1.26–3.46; p = **0.005**) (Thakur *et al*., 2010).

A study conducted by Shukla et al. examined the association of genetic polymorphism of *GSTT1, GSTM1,* and *GSTM3* in COPD patients in a North Indian population. The genotypes of 412 individuals (204 COPD patients and 208 healthy people) were studied. The frequency

of homozygous *GSTM1* null genotype was found to be significantly higher in COPD patients than in healthy controls (OR= 2.58; 95% CI= 1.73–3.84; p= **0.001**), but there was no correlation in the distribution of homozygous null *GSTT1* and 3-bp deletion polymorphism (rs1799735) in intron six variant allele in *GSTM3* between COPD patients and healthy subjects (Shukla *et al*., 2011).

The research carried out by Zuntar *et al.* determined the association of GST polymorphism with COPD. *GSTP1*, *GSTM1*, and *GSTT1* genotypes were ascertained using DNA methods, and GST activity was measured spectrophotometrically in older male Caucasian Croats (non-smokers, ex-smokers, and smokers) with stable COPD (n = 30) and sex/age-matched controls (n = 60). The *GSTP1* mutant genotype of exon 5 (GG), as well as *GSTP1* mutant and heterozygous genotypes of exon 6 (TT and CT), were found to be genetic contributors to COPD susceptibility, whereas null *GSTM1*, null *GSTT1*, joint *GSTM1/GSTT1* null genotypes, and wild type *GSTP1* genotypes (AA and CC) were not. There was no effect of GST genotypes on serum GST activity in COPD patients, and controls were divided by smoking history (Zuntar *et al*., 2014).

A report by Dey *et al*. analyzed the role of Glutathione S Transferase Polymorphism in COPD with particular emphasis on people living near an open cast coal mine site in Assam. PCR-based methods were used to determine the genotypes of 70 COPD patients and 85 non-COPD patients, followed by multiplex PCR of the *GSTT1* and *GSTM1* genes, with the albumin gene serving as an internal control. Suspended particulate analyses were performed using the Respirable Dust sampler and FTIR analysis of dust specimens taken from glass microfiber filters. The analysis of dust samples reveals a higher level of respirable suspended particulate matter, non-respirable particulate matter, SO_2, and NO_2 in the study site's air. FTIR analysis also indicates a higher concentration of organic silicone and aliphatic C-F

compounds in the study site's atmosphere. When spirometry was performed, most subjects had low lung function (Dey *et al*., 2014)

El Wahsh *et al*. analyzed the relation between COPD susceptibility and polymorphisms of Glutathione S-transferases (GST); *GSTM1, GSTT1,* and Microsomal epoxide hydrolase-1 (*EPHX1*) genes in the Egyptian population. The genetic polymorphisms of the *GSTM1*, *GSTT1*, and *EPHX1* genes were studied in 146 COPD patients and 130 controls using multiplex PCR for the *GSTM1* and *GSTT1* genes and PCR-RFLP for the *EPHX1* genes. *GSTM1*-null genotype was more common in patients compared to controls (72.6% versus 43.8%, p<**0.001**). Carriers including null *GSTT1* and *GSTM1* genes were more likely to develop COPD (OR= 3.45, 95% CI = 1.07–11.14). Patients had a higher *EPHX1* exon 3 His allele frequency than controls (19.2 % versus 12.7%, p = **0.04**). Carriers of the exon 3 His allele were more likely to develop COPD (OR= 1.63, 95 percent CI = 1.02–2.6, P = **0.04**). Carriers with both *GSTM1*-null and *EPHX1 113Tyr/Tyr* or *EPHX1 113Tyr/His* genotypes had a greater risk of COPD (OR= 3.33, 95% CI = 1.32–8.35 and OR=14.24, 95% CI = 3.02–67.17). Carriers with *GSTM1*-null and *EPHX1 139His/His* genotypes were more likely to experience COPD (OR =5.58, 95%CI = 2.14–14.52) (El Wahsh *et al*., 2015).

A case-control study by Khan *et al*. investigated the association between xenobiotic-metabolizing gene polymorphism and COPD in the Indian population. The study included 186 COPD patients and 160 healthy subjects. *GSTT1* and *GSTM1* allele frequencies were deduced using conventional multiplex PCR, while *GSTP1* was determined using polymerase chain reaction and restriction fragment length polymorphisms. There was a significant case-control difference in the presence of null *GSTM1* (61.8% vs 55.0%, p=0.04). There was no correlation between *GSTT1* null genotype and COPD susceptibility (54.8 %vs 50.6% OR= 1.26; CI= 0.87-1.84, p=0.82). They discovered that subjects who were homozygous for the *GSTP1* polymorphism *Val/Val* were at an increased risk of COPD development (OR= 2.58,

95 % CI=1.2-4.8) compared to heterozygote variants *Ile/Val* (OR: 1.28 CI: 0.7-2.14). Furthermore, the mutant allele frequency (Val) in patients was significantly higher than in controls, and the difference was significant. (OR = 1.8, 95% CI = 1.4-4.2, p=**0.001**) (Khan *et al*., 2016).

The investigation done by Cao *et al*. assessed the effects of Glutathione S-Transferase gene polymorphisms and antioxidant capacity per unit albumin on COPD pathogenesis. *GSTM1* and *GSTT1* gene polymorphisms were observed using polymerase chain reaction technology in COPD patients and healthy individuals. The I_2/KI potentiometric, H_2O_2 potentiometric, and $KMnO_4$ microtitration methods evaluated the total antioxidant capacity (TAC). The AC/ALB ratio was calculated by dividing the TAC by the serum albumin concentration. Logistic regression analysis was performed on biochemical screening indices closely related to COPD incidence. The COPD patients had a significantly higher *GSTM1* and *GSTT1* gene deletion rate than the control group (p<**0.05**). There were statistically significant differences in serum TAC between the COPD and healthy controls, *GSTM1* (+) and *GSTM1* (-) groups, and *GSTT1* (+) and *GSTT1* (-) groups (p<**0.001**). Besides that, there was a statistically significant difference in AC/ALB between the COPD and control groups (p<**0.05**). The occurrence of COPD was closely related to the AC/ALB (p<**0.05**) using logistic regression analysis. *GSTM1* and *GSTT1* gene polymorphisms are linked to the pathogenesis of COPD, whereas the AC/ALB plays an essential role in the onset and progression of COPD (Cao *et al*., 2017).

The meta-analysis by Ding *et al*. attempted to link glutathione S-transferase gene *M1* and *T1* polymorphisms and COPD risk. Thirty-seven articles were screened, with 4674 COPD patients and 5006 controls included. *GSTM1* and *GSTT1* null genotypes were found to significantly increase the COPD risk (*GSTM1*: OR=1.52, 95% CI=1.31-1.77, p<**0.00001**; *GSTT1*: OR=1.28, 95% CI=1.09-1.50, p=**0.003**). Furthermore, they discovered that combined

GSTM1/GSTT1 null genotypes were also associated with a higher risk of COPD (OR=1.42, 95%CI=1.21-1.66, p<**0.0001**) (**Table 2.2**) (Ding *et al*., 2018).

Table 2.2: Ethnic analysis of the association of *GSTM1*, *GSTT1*, and combined *GSTM1/GSTT1* null genotype in COPD susceptibility.

GST variant	Ethnicity	Odds Ratio	95% Confidence Interval	p-Value
GSTM1	Asians	1.59	1.29-1.196	**0.0001**
	Caucasians	1.27	1.11-1.44	**0.0004**
	Africans	2.42	1.36-4.31	**0.003**
GSTT1	Asians	1.47	1.21-1.78	**0.0001**
	Caucasians	1.07	0.70-1.62	0.75
	Africans	1.53	1.06-2.20	**0.02**
GSTM1/GSTT1	Asians	1.30	1.05-1.61	**0.02**
	Caucasians	1.28	0.89-1.86	0.18
	Africans	1.18	0.87-1.62	0.29

The above-published studies emphasized the critical role of GSTs (especially *GSTM1*&*GSTT1*) in COPD susceptibility. However, the results are contradictory, which needs to be clarified with extensive case-control matched investigations to establish an appropriate genetic marker to diagnose this disease and prevent lung deterioration.

2.7 Inflammation

Inflammation is a protective response of the immune system intended to protect the human body from harmful stimuli such as toxins, pathogens, irradiation, or damaged cells and start the healing process. Inflammation is thus an essential defence mechanism for health, but it can be deleterious if it leads to structural changes and tissue damage (Laroux *et al*.,2004) (American Thoracic Society). Acute inflammation, a regulated type of inflammation, and chronic inflammation, a dysregulated form of inflammation, are the two types. The removal of infectious agents is accompanied by a resolution and repair process induced primarily by tissue-resident and selected macrophages in an effective acute inflammatory response (Serhan *et al*.,2005). If the critical inflammatory response does not eliminate the pathogen, the inflammatory process continues and takes on new characteristic features. In the case of infection, macrophages and T cells replace neutrophil infiltration. If the cumulative impact of these cells is still inadequate, a chronic inflammatory state with granulomas and tertiary lymphoid tissues develops (Drayton *et al*.,2006).

2.8 COPD pathology

COPD is defined by the development of excessive chronic inflammation in the lungs in reaction to cigarette or tobacco smoke, compared to smokers that do not have lung disease (Hogg *et al*., 2009). The narrowing of the small airways, remodelling, and obliteration of the lung parenchyma with the loss of the alveolar attachments of these air passages is an outcome of emphysema resulting in gradual airway obstruction in patients with COPD (Barnes *et al*., 2016). These pathological changes appear as a result of chronic inflammation in the lung periphery, which intensifies as the disease advances (Hogg *et al*., 2004). Even in people with mild disease, the peripheral airways are obstructed and lost (McDonough *et al*., 2011). The

site of accumulated toxic particles, such as cigarette and wood smoke, is thought to reflect the peripheral location of inflammation in COPD individuals (Barnes *et al.*, 2016).

2.8.1 Methodologies for phenotyping COPD airway inflammation and damage

Lung specimens acquired during the surgical intervention and post-mortem have provided insights into respiratory inflammation and damage. Notably, *in vivo* measures of the airway and systemic inflammation have been distinguished longitudinally, at exacerbations, and in response to therapies, using lung imaging (small airways indirectly and large airways directly); invasive sampling of the airway by bronchoscopy (smaller airways by bronchoalveolar lavage and large airways by brush and biopsy); breath analysis (small and large airways) (Brightling *et al.*,2019); non-invasive sputum sampling (mainly large airways), which is safe even in severe COPD (Brightling *et al.*, 2001) and even beyond the lung by examining upper airway samples and using urine and blood systemically (George *et al.*, 2016; Brightling *et al.*, 2016) (**Fig 2.6**)

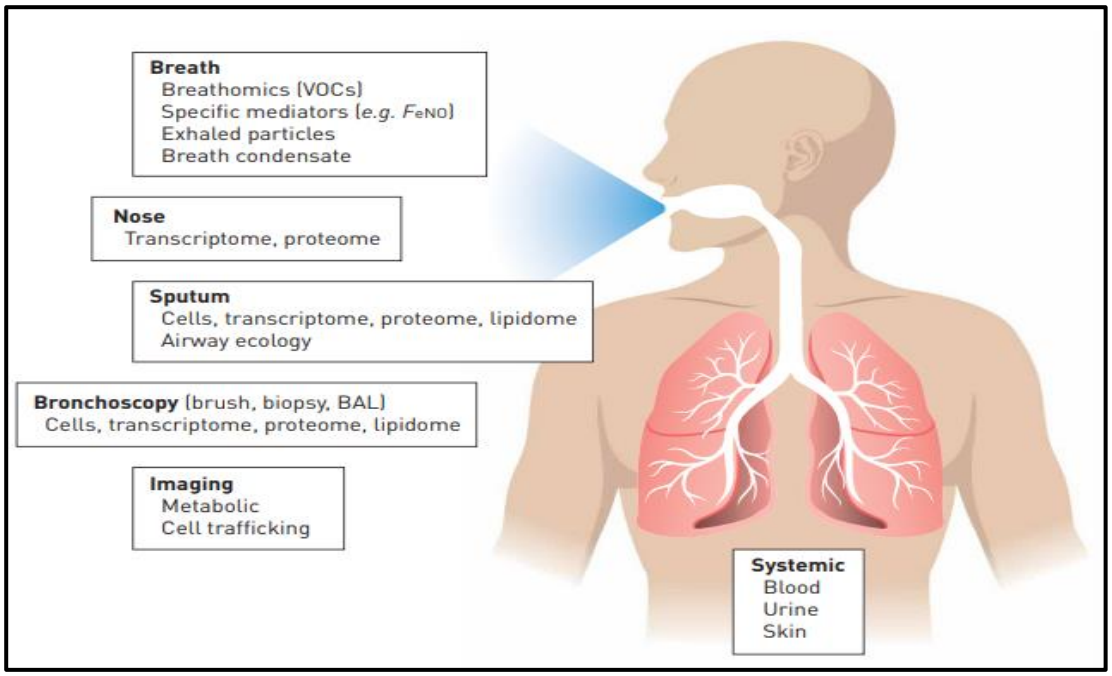

Figure 2.6: Sampling approaches to phenotype airway inflammation and damage in COPD (Brightling*et al.*,2019)

2.8.2 Characteristics of COPD-related inflammation

There is a distinct pattern of inflammation in COPD patients, with increased numbers of macrophages and neutrophils in the lumen of the airway, T lymphocytes, and B lymphocytes (Brusselle *et al*., 2011). The inflammatory response in COPD patients includes innate and adaptive immune responses associated with dendritic cell activation (Givi *et al*.,2012). A comparable inflammation and mediator expression pattern are observed in smokers without airway obstruction. Still, this inflammation is amplified in COPD patients and even further increased during acute exacerbations or when a bacterial or viral infection is present (Barnes *et al*., 2016) (**Figure 2.7**).

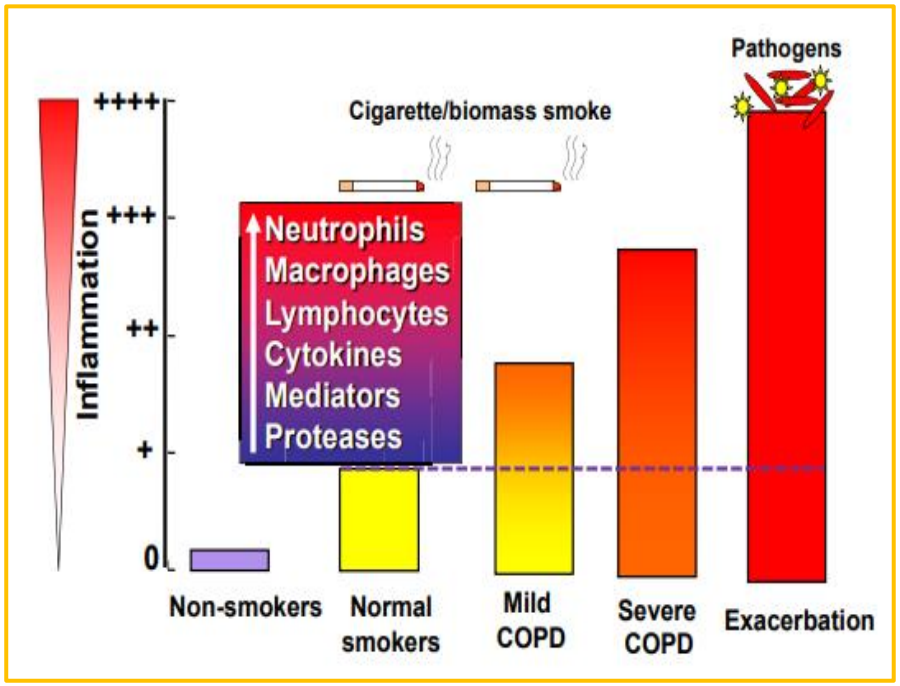

Figure 2.7: Graphical illustration of amplified inflammation in COPD patients compared to smokers without airflow limitation (Barnes et al., 2016)

The molecular mechanisms for the amplification of inflammation are unknown, but genetic and epigenetic factors may play a significant role. The molecular mechanisms of amplification may play a part in influencing which smokers are at risk of developing airway occlusion. Inhaled cigarette smoke and other irritants may cause surface macrophages

and airway epithelial cells to generate a variety of chemotactic mediators, including chemokines, which allure was circulating neutrophils, monocytes, and lymphocytes into the lungs (Barnes *et al*., 2016). This inflammation endures even after smoking cessation, implying that self-perpetuating pathways have yet to be discovered (Gamble et al., 2007). Long-lived memory T cells, bacterial colonization, or autoimmunity could contribute to the persistent inflammation observed in COPD patients (Barnes *et al*., 2016)

2.8.3 Inflammatory cells in COPD

COPD causes many inflammatory cells to be recruited from the blood into the lungs by chemotactic factors released locally. Epithelial cells, endothelial cells, and fibroblasts are structural cells in the lungs that release inflammatory mediators and participate actively in the inflammatory process. In COPD, innate immunity [eosinophils, neutrophils, macrophages, mast cells, natural killer cells, γ δ -T cells, innate lymphoid cells (ILCs), and DC], as well as adaptive immunity (T and B lymphocytes), are involved in the inflammatory response (Barnes *et al*., 2017) (**Figure 2.8**).

Macrophages

Blood monocytes, which commute to the lungs and differentiate locally, are the primary source of macrophages. Macrophages differ in their inflammatory profiles, some being pro-inflammatory, while others are anti-inflammatory, promote tissue repair, and are more phagocytic. The inflammatory response appears to be orchestrated by macrophages. Macrophages play a crucial role in COPD pathophysiology, accounting for most of the disease's known symptoms. In patients with COPD, the number of macrophages in the airways, lung parenchyma, BAL fluid, and sputum increases dramatically (5– 10-fold). In patients with emphysema, macrophages are also found near sites of alveolar wall destruction. There is a link between the number of airway macrophages and COPD severity. Cigarette

smoke extract may activate macrophages, causing them to release inflammatory mediators such as tumour necrosis factor (TNF-), IL-8, other CXC chemokines, and monocyte chemotactic peptide (MCP)-1, LTB4, and reactive oxygen species, establishing a cellular mechanism that relates smoking to COPD inflammation. Elastolytic enzymes secreted by alveolar macrophages include MMP-2, MMP-9, MMP-12, cathepsins K, L, and S, and neutrophil elastase. Nuclear factor-B (NF-B), activated in alveolar macrophages of COPD patients, mainly during exacerbations, regulates most of the inflammatory proteins that are upregulated in COPD macrophages. Increased monocyte recruitment from the circulation in response to monocyte selective chemokines may explain the increased numbers of macrophages in COPD patients and smokers. Macrophages can also release the chemokines interferon-c inducible protein (IP10), interferon-inducible T-cell chemoattractant (I-TAC), and monokine-induced by interferon-c (Mig), which are chemotactic for CD8z Tc1 cells when they interact with the CXCR3 receptor. Increased macrophage numbers in COPD may be due to increased monocyte recruitment, but they could also be due to increased proliferation and more prolonged lung survival. CXCL9, CXCL10, and CXCL11, chemotactic for $CD8^{+}$ cytotoxic T (Tc1) cells and CD4 + Th1 cells, are also released by macrophages when they interact with the chemokine receptor CXCR3 on these cells. COPD patients' macrophages produce more inflammatory proteins than average smokers and non-smokers' macrophages, indicating increased activation (Rovina *et al*.,2013).

Neutrophils

COPD is characterized by neutrophilic inflammation, with increased numbers of activated neutrophils in sputum and BAL fluid, which correlates with disease severity. Due to their rapid transit into the lumen, few neutrophils are seen in the airway wall and lung parenchyma. Smoking imparts a direct stimulatory effect on granulocyte production, release from the bone marrow, and survival in the respiratory tract, which may be mediated by the

hematopoietic growth factors GM-CSF and G-CSF released from airway epithelial cells and lung macrophages. In mice, an anti-GM-CSF antibody reduces lung neutrophilic inflammation after exposure to cigarette smoke. The recruitment of neutrophils to the airways and parenchyma begins with their adhesion to endothelial cells via E-selectin, which is upregulated on endothelial cells in COPD patients' airways. Various neutrophil chemotactic factors such as LTB4, CXCL1, CXCL5 (ENA-78), and CXCL8 that are increased in COPD airways direct adherent neutrophils into the respiratory tract. These chemotactic mediators can come from various sources, including epithelial cells, macrophages, and T cells, but neutrophils may be the primary source of CXCL8. Increased secretion of granule proteins such as myeloperoxidase (MPO) and human neutrophil lipocalin by neutrophils recruited to COPD patients' airways activates them. Serine proteases, such as cathepsin G, neutrophil elastase (NE), and proteinase-3, as well as MMP-8 and MMP-9, are secreted by neutrophils and may contribute to alveolar destruction. Because NE, cathepsin G, and proteinase-3 are potent stimulants of mucus secretion from submucosal glands and goblet cells, airway neutrophilia is linked to mucus hypersecretion. In acute exacerbations, the number of neutrophils in the airways increases, which accounts for the increased purulence of sputum (Barnes *et al*., 2017).

Lymphocytes

Lymphoid follicle development in the small airway walls as COPD progresses is a distinguishing feature. T and B cells make up these lymphoid follicles. T cells are also present in the parenchyma and airways of the lungs, with studies showing that CD8+ cells outnumber CD4+ cells. The severity of airway obstruction and alveolar destruction are related to the number of T cells. The main difference between asymptomatic smokers and smokers with COPD is an increase in T cells, particularly Tc1 cells, in patients with COPD, suggesting that they may play a significant role in amplifying and maintaining inflammation.

Smokers with COPD have more Th1 cells in their airways, expressing activated STAT-4, a transcription factor required for the activation and commitment of the Th1 lineage. Th17 cells that secrete IL-17A and IL-22 are also found in higher numbers in COPD patients' airways and may help orchestrate neutrophilic inflammation. IL-6 and IL-23 released by alveolar macrophages may regulate Th17 cells. COPD patients' CD4 + and CD8 + T cells express higher levels of CXCR3, a receptor for CXCL9, CXCL10, and CXCL11, which are increased in COPD. CXCL10 expression is higher in bronchiolar epithelial cells, which may contribute to the accumulation of CD4 + and CD8 + T cells, which preferentially express CXCR3.CD8+ cells are generally increased in response to infections, so COPD patients' chronic bacterial colonization of the lower respiratory tract may be causing this inflammatory response. In emphysema, there is a link between CD8+ cells and alveolar cell apoptosis. CD8+ cells are cytotoxic and induce apoptosis by secreting perforins, granzyme B, and TNF. Increased numbers of T cells with no expression of the co-stimulatory receptor CD28 (CD4/CD28null, CD8/CD28null cells) release increased amounts of perforins, and granzyme B is evidence of immunosenescence in COPD. IL17A is elevated in end-stage COPD, according to a study, and this contributes to cigarette smoke-induced lymphoid neogenesis (Barnes *et al*., 2016).

2.8.4 Other inflammatory cell types involved in COPD

Eosinophils

Even though eosinophils are the most common type of leukocyte in asthmatic patients, their role in COPD patients is less clear. According to some studies, eosinophil numbers are higher in the airways and BAL fluid of patients with stable COPD but not in airway biopsy specimens, BAL fluid, or induced sputum. Although neutrophil-associated COPD is the most common inflammatory phenotype, 10–40% of COPD patients have increased eosinophilic inflammation in their sputum and blood, as well as increased T2-transcriptome signatures,

demonstrating the disease's heterogeneity. In COPD, as in asthma, increased eosinophilic inflammation in peripheral blood and sputum samples are linked to a higher risk of severe exacerbations in the future. Eosinophilic inflammation in COPD has an unknown cause. Eosinophilic COPD, like neutrophil-associated COPD, is likely due to a combination of innate and adaptive immunity. For asthma, these pathways have been well described. Th2 cells produce IL-4, IL-5, and IL-13 after allergic sensitization and T-cell polarization. IL-5 is required for eosinophil survival and maturation, while IL-4 and IL-13 promote IgE production from B cells and directly affect structural cells. Eosinophil recruitment to the lung mucosa is mediated by the production of CCR3 chemokines, primarily produced by epithelial cells, and other eosinophil chemo attractants, such as mast cell-derived prostaglandin (PG)D2. PGD2 activates PGD2 type 2 receptors, boosting T2 immunity (DP2 or CRTH2). Even though atopy is not increased in eosinophilic COPD, total IgE is elevated. ILC2 cells, which generate IL-5 and IL-13 in response to PGD2 and the epithelial-derived "alarmins" IL-33, IL-25, and TSLP released after epithelial damage by pollutants and microbes, can also cause eosinophilic inflammation. IL-33 produced by macrophages and released after inflammasome activation could also play a role (Brightling *et al*., 2019).

Epithelial cells

Cigarette smoke and other inhaled toxins, such as biomass fuel smoke, cause epithelial cells to produce inflammatory mediators such as TNF-a, IL-1b, IL-6, GM-CSF, and CXCL8 (IL-8). TGF-b is produced by epithelial cells in the small airways, which causes local fibrosis. The vascular endothelial growth factor (VEGF) appears to be required for alveolar cell integrity, and blocking VEGF receptors in rats induces alveolar cell apoptosis and an emphysema-like pathology. Although smokers and COPD patients have lower levels of VEGF in their peripheral lungs, smokers have higher levels of another growth factor, hepatocyte growth factor, which may protect against the effects of lower VEGF levels on

alveolar integrity. Both VEGF and hepatocyte growth factor levels are reduced in COPD patients, which may contribute to the development of emphysema. With mucus production from goblet cells and secretion of antioxidants, antiproteases, and defensins/cathelicidins, airway epithelial cells play an essential role in airway defence. Cigarette smoke and other noxious agents may impair the airway epithelium's responses, increasing infection susceptibility. Patients with COPD have higher levels of epithelial growth factor receptors (EGFRs), which may contribute to basal cell proliferation, resulting in squamous metaplasia and an increased risk of bronchial carcinoma (Barnes *et al*., 2017).

Inflammatory mediators

Lipids, free radicals, cytokines, chemokines, and growth factors are some inflammatory mediators linked to COPD. 80 These mediators are made up of inflammatory and structural cells in the lungs and interact in a complicated way. Because so many mediators are involved, blocking just one is unlikely to have a significant clinical effect. Similar mediators found in the lungs of COPD patients may be elevated in the circulation, and this systemic inflammation may underpin and exacerbate comorbidities. Cytokines, reactive oxygen species, and proteases have all been linked to pathogenic inflammation in COPD. The activation of Toll-like receptors (TLRs) and lymphocyte antigen receptors leads to the release of inflammatory mediators via intracellular signalling pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-) and signal transducers and activators of transcription (STATs) (Barnes *et al*., 2016).

Cytokines

Macrophages and T cells are the leading producers of cytokines in the lungs. Because different techniques were used and studies reported baseline levels and responses to stimulation, a wide range of outcomes were reported between studies. Tumour necrosis

factor-alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-8 are important mediators produced by macrophages and neutrophils. In COPD, these cytokines are notably pro-inflammatory. Interferon-gamma (IFN-γ) and tumour necrosis factor (TNF- α) levels are elevated in COPD, particularly in the baseline state, consistent with Th1 responses. Both of these cytokines are linked to active inflammation. Intriguingly, both IFN- γ and IL-13 (Th2) models have been related to emphysema development in mice .IL-17 levels are elevated in COPD patients, according to recent publications (Rovina *et al*., 2013).

Chemokine

Several chemokines have been linked to COPD in patients, which has intrigued researchers because chemokine receptors are G protein-coupled receptors for which small molecule antagonists have been developed. CXCL8 concentrations rise in COPD patients' induced sputum and rise even higher during exacerbations. CXCL8 is produced by macrophages, T cells, epithelial cells, and neutrophils and acts as a chemotactic chemokine for neutrophils via the high-affinity CXCR2, which is also activated by related CXC chemokines like CXCL1. CXCL1 levels are significantly higher in COPD patients' sputum and BAL fluid, suggesting that this chemokine may be essential as a chemoattractant than CXCL8, which acts through CXCR2, which is expressed on neutrophils and monocytes. CXCL1 causes significantly more chemotaxis in COPD patients' monocytes than in regular smokers, possibly due to increased turnover and recovery of CXCR2. During COPD exacerbations, CXCL5 expression in airway epithelial cells increases dramatically, accompanied by a significant upregulation of epithelial CXCR2. CCL2 is found in higher concentrations in COPD patients' sputum and BAL fluid, and it plays a role in monocyte chemotaxis by activating CCR2. CCL5 (RANTES), a chemokine that activates CCR5 on T cells and CCR3 on eosinophils, is also expressed in the airways of COPD patients during exacerbations, which could explain the increased eosinophil and T-cell numbers seen in the walls of large airways during

exacerbations of chronic bronchitis. CXCR3 expression is upregulated in COPD patients' TC1 and TH1 cells, along with increased expression of the ligands CXCL9, CXCL10, and CXCL11. Increased chemotaxis of monocytes and lymphocytes from COPD patients is induced by 66 CXCR3 ligands, possibly due to increased CXCR3 expression in COPD patients (Barnes *et al*., 2008).

Reactive oxygen species

Excessive reactive oxygen species production damages lung tissue and causes oxidative stress, a primary pathogenic process in COPD. ROS are produced by phagocytes (neutrophils and macrophages) and epithelial cells, and this process is accelerated in COPD patients. To counteract the effects of ROS, antioxidants such as superoxide dismutase (SOD), glutathione, and catalase are produced and regulated by the nuclear erythroid-2-related factor-2 (Nrf2). The activation of Nrf2 is deficient in COPD patients. The pro-inflammatory effects of ROS are numerous. Inflammatory transcription pathways are also activated by ROS. Oxidative stress inhibits the activity of sirtuin-1, a protein involved in tissue repair, as well as growth factors and DNA damage. COPD patients' lungs age more quickly due to oxidative stress (Malhotra *et al*.,2008).

Proteases

Neutrophils and macrophages both produce proteases. Neutrophil elastase and matrix metalloproteinases (MMP) 9 and 12 are two proteases. Proteases are essential factors in the progression of emphysema. Anti-proteases, such as α-1 antitrypsin, counteract their effect. A lack of α-1 antitrypsin can cause emphysema to develop quickly and severely. They also contribute to neutrophil-mediated inflammation and increased arterial stiffness. A diverse range of stimuli (including cytokines, ROS, oxidized low-density lipoprotein, and bacterial antigens) bind to surface receptors (e.g., Toll-like receptors and antigen receptors) on

immune cells, activating intracellular signalling pathways that drive the inflammatory response. NF-κβ and STATs are two crucial intracellular signalling pathways relevant to COPD (Caughey *et al.*,2016).

Lipid mediators

The profile of lipid mediators in COPD patients' exhaled breath condensates shows an increase in prostaglandin (PG) and long-chain triglycerides (LT). PGE2 and PGF2a, and LTB4 concentrations have increased significantly, but cysteinyl leukotrienes have not. 81 This is not the same pattern as in asthmatic patients with higher levels of thromboxane and cysteinyl leukotriene. During acute exacerbations, LTB4 concentrations rise in induced sputum and even higher in sputum and exhaled breath condensate. LTB4 is a potent neutrophil chemoattractant that works through high-affinity BLT1 receptors. The neutrophil chemotactic activity of the sputum is reduced by about 25% when a BLT1 receptor antagonist is used. BLT1 receptors have been discovered on T lymphocytes, and there is evidence that LTB4 is also involved in T cell recruitment (Barnes *et al*., 2016).

Inflammasome

Inflammasomes are multi-protein signalling complexes that help regulate the expression of pro-inflammatory cytokines IL-1, IL-1, and IL-18 by releasing them from precursors via caspase-1 generation in response to external and endogenous stimuli danger signals. NLRP3 inflammasomes have received the most attention, as they may play a role in various inflammatory lung diseases, including asthma and COPD. The adapter, ASC (apoptosis-associated speck-like protein containing a CARD), which recruits pro-caspase-1 to the protein complex and is increased in the lungs of COPD patients, is a vital component of the NLRP3 inflammasome. ASC accumulation is linked to the formation of extracellular' specks,' which help maintain IL-1β production outside the cell. There is no increase in NLRP3

inflammasomes in patients with stable COPD, except in patients with severe disease, possibly due to the inflammasome inhibitory molecules NALP7 and IL-37. The lack of benefit with an IL-1β blocking antibody (canakinumab) in stable COPD underscores the inflammasome's minor role in COPD. As with asthma, NLRP3 inflammasome activation is more likely linked to acute exacerbations, where pathogens, oxidative stress, and ATP, all of which activate the NLRP3 inflammasome, are elevated (Barnes *et al*., 2017).

Dendritic cells

DC are specialized macrophage-like cells in the airway epithelium that serve as the primary antigen-presenting cells in the airways and as a crucial link between innate and adaptive immunity in the lungs. DC absorb allergens, convert them to peptides, and then deliver the allergenic peptides to uncommitted T lymphocytes to program the production of allergen-specific T cells. Immature DC in the respiratory tract promotes helper T-cell (Th2) cell differentiation. In COPD patients' lungs, DC are activated and increased in number, especially in severe disease (Rovina *et al*., 2013).

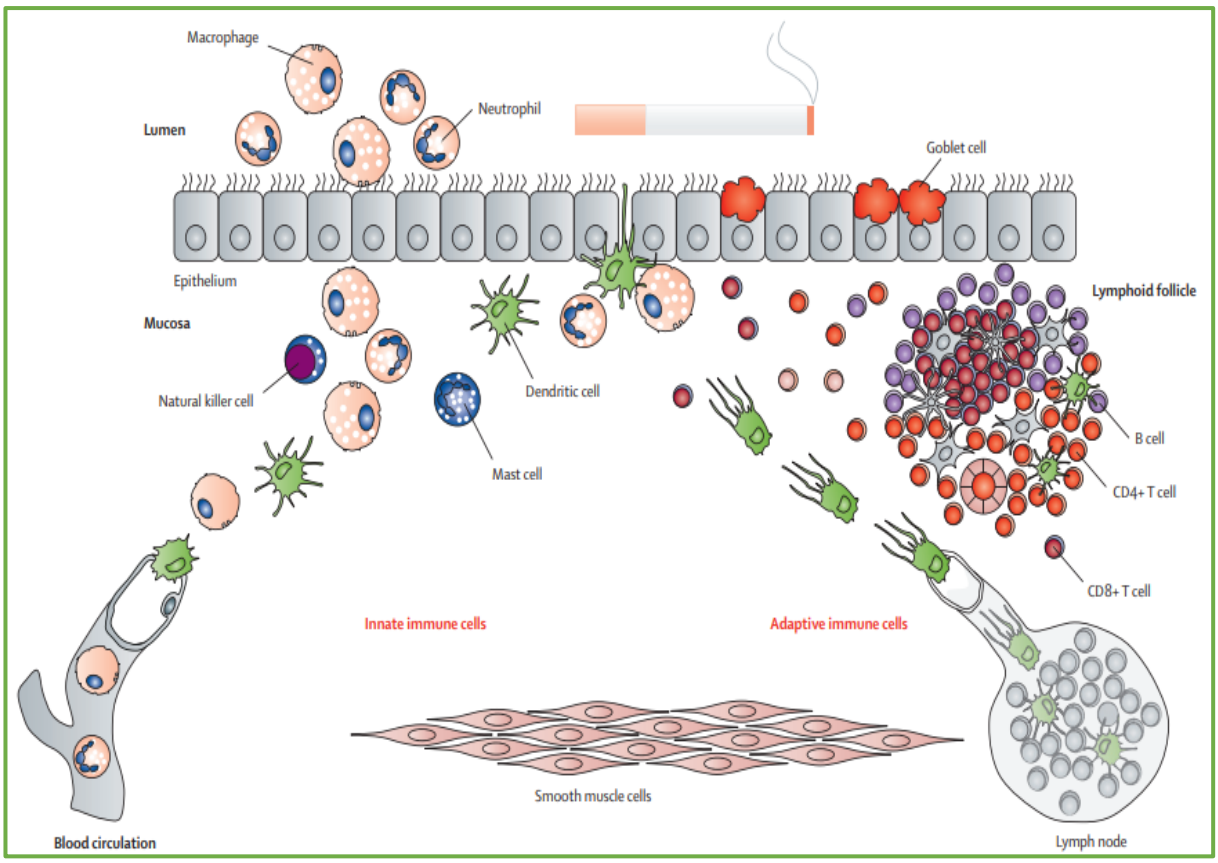

Figure 2.8: Illustration of involvement of Innate and adaptive immune cells in the COPD pathogenesis (Brusselle *et al*.,2011)

Systemic inflammatory response

Chronic obstructive pulmonary disease is linked to a solid systemic immune response, becoming more pronounced as the disease signs progress and exacerbations occur. Compared to smoking controls, systemic inflammation is defined as inflammatory/immune response mediators in the peripheral blood with elevated levels of COPD (without COPD). Systemic inflammation, measured by biomarkers such as C-reactive protein (CRP), leukocytes, and fibrinogen, is related to a two- to four-fold increased risk of comorbidities such as heart disease and lung cancer. Another study found elevated levels of at least one inflammatory marker (CRP, IL-6, IL-8, TNF-, fibrinogen, and leukocytes) in 70% of patients and persistent inflammation in 16%. Many studies have described systemic inflammation in COPD, but the definition of this entity has often been ambiguous. It is unclear whether the systemic

inflammatory response is primarily a separate systemic component of the disease or a spillover of lung mediators. Because of their proximity to the lung, complications like osteoporosis, cerebrovascular disease, and muscle wasting are more relevant than lung cancer and coronary artery disease (Barnes *et al.*, 2016)

2.9 IL-4

Basophils, mast cells, a subset of activated T cells, eosinophils, and neutrophils are the leading producers of interleukin-4 (IL-4) (Chomarat and Banchereau *et al*.,1997). When naive peripheral CD4+ T cells are activated, they begin synthesizing and secreting cytokines. As a result of these cytokines acting as autocrine growth and differentiation factors, immature T cells proliferate and differentiate into effector cells. Different subsets of effector helper T (Th) cells can be distinguished based on the pattern of cytokines they secrete. Th1 cells produce IL-2, interferon-gamma (IFN-), and tumour necrosis factor (TNF), whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-13. The polypeptide IL-4 has a length of 15 kD. Its receptor is a heterodimer composed of an IL-4-binding subunit and a common subunit found in other cytokine receptors (Choi *et al*., 1998). The binding of IL-4 to its receptor causes T cells to proliferate and differentiate into Th2 cells (Paul *et al.*,2007). IL-4, a pleiotropic cytokine, is crucial in immune response shaping (Gessner *et al.,* 2000). It is also called BSF or BCGF1. This cytokine is an interleukin four receptor ligand. The interleukin four receptor binds to IL13, which may contribute to the cytokine's and IL13's many overlapping functions. STAT6, a signal transducer and transcription activator, has been shown to play a vital role in mediating this cytokine's immune regulatory signal. On chromosome 5q, this gene, along with IL3, IL5, IL13, and CSF2, forms a cytokine gene cluster, with this gene being particularly close to IL13. Several long-range regulatory elements in an over 120-kilobase range on the chromosome are found to regulate this gene, IL13 and IL5. IL4 is a cytokine that

aids tissue repair by counteracting the effects of pro-inflammatory type 1 cytokines, but it also promotes allergic airway inflammation.

Furthermore, as a type 2 cytokine, IL-4 mediates and regulates a wide range of human host responses, including allergic, anti-parasitic, wound healing, and acute inflammation. This cytokine has been shown to help neutrophil-mediated acute lung injury heal faster. IL-4 is required to produce allergen-specific immunoglobin (Ig) E during an allergic reaction. IL4 gene has two alternatively spliced transcript variants that encode different isoforms (NCBI).

2.9.1 Association of IL-4 and COPD

The cytokine IL-4 plays a key mediator role in the intercellular signalling network, acting in allergic immune responses to allergen exposure (Vitenberga *et al*., 2019). Luzina *et al*. (2015) found that IL-4 affects mucosal connective tissue fibroblasts and promotes local fibrosis. Increased mucus production has been linked to increased cytokine IL-4 release from immune and non-immune cells in COPD [Boer *et al*.,2007].

Hegab *et al*. studied two ethnic groups: Japanese (88 COPD patients and 61 control subjects) and Egyptian (88 COPD patients and 61 control subjects) (106 patients with COPD and 72 control subjects). IL4 polymorphisms -589 C/T, -33 C/T, and a variable number of tandem repeat (VNTR) were genotyped, IL13 polymorphisms - 1111 C/T and + 2044 G/A were genotyped, and ADRB2 polymorphisms +46 A/G and + 79 C/G were genotyped. The frequencies of pairwise haplotypes, as well as genotype and allele frequencies, were studied. In Egyptians, ADRB2 + 79 C/G genotype frequencies significantly differed between the COPD and control groups (p=**0.002**). The haplotype distributions in the Japanese (IL4 - 589 C/T: IL4 VNTR; IL4 - 33 C/T: IL4 VNTR) and Egyptians (IL4 - 589 C/T: ADRB2 + 79 C/G; IL4 VNTR: ADRB2 + 79 C/G) [corrected p values, **0.033** and **0.001**, respectively] showed significant differences between the COPD and control groups. The ADRB2 + 79 C/G

polymorphism and the haplotypes identified in this study were thought to play a role in COPD pathogenesis (Hegab *et al*.,2004).

Shukla et al. observed the allele and genotype distributions of IL1B, IL1RN, TNF-, and IL4 in COPD patients (N=204) and healthy people (N=208) in a study. Whole blood was used to obtain genomic DNA, which was then genotyped using a PCR-based Restriction Fragment Length Polymorphism technique. The *IL1RN*2/IL1RN*2* genotype was protective for male COPD patients, with a frequency of 8.7% in COPD patients and 14.6% in healthy subjects (p=**0.017**; OR=0.53), but the IL1RN*1/IL1RN*2 genotype was found to be a risk factor for female COPD patients. There were insignificant differences in genotype frequencies of the polymorphisms T (-511) C of IL1B and 70bp VNTR of IL-4 between COPD patients and healthy subjects. The TNF-polymorphism GA has genotype G (-308) A allele was significantly associated with COPD patients (p=0.023; OR=0.65), and it was more common in COPD patients than in controls (20.5 % vs 14.4 %; p=0.107) (Shukla *et al*.,2012).

A study by Huang *et al*. depicted the levels of plasma inflammatory cytokines and how they relate to pulmonary function in patients with asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS). Ninety-six patients with asthma, acute exacerbation of chronic obstructive pulmonary disease (AECOPD), or ACOS were enrolled, with a control group of 35 healthy people. The levels of interleukin (IL)-4, IL-8, IL-10, and tumour necrosis factor-alpha (TNF-a) in fasting plasma were determined (ELISA). The researchers looked at the relationships between plasma inflammatory cytokine levels and FEV1, FEV1/predicted value ratio (FEV1% pred), and FEV1/forced vital capacity (FVC). The three groups of patients had statistically substantial differences in IL-4 and IL-8 levels (p<**0.001**). The AECOPD and ACOS groups had significantly lower IL-4 levels, while the asthma group had significantly higher IL-8 levels (p<**0.05**). The three patient groups had very different levels of IL-10 and TNF-a (both p<**0.001**). Each of the two groups had quite different levels of IL-10

(all p<**0.001**). TNF-a levels were higher in the asthma group than in the AECOPD and ACOS groups (P<**0.001**). FEV1, FEV1 % pred, and FEV1/FVC were positively related to IL-4 and IL-10, while IL-8 and TNF-a were negatively correlated. Inflammatory cytokines IL-4, IL-8, IL-10, and TNF-a, are linked to the severity of airway diseases and could be used as diagnostic markers for asthma, COPD, and ACOS (Huang *et al.*,2016).

In a case-control study, Chi et al. found a link between IL-4 gene polymorphisms and chronic obstructive pulmonary disease (COPD) in Hebei Province patients. Blood samples were taken from 62 COPD patients and 301 healthy people. PCR with sequence-specific primer typing was used to detect polymorphisms in the *IL-4* gene. Between COPD patients and healthy subjects, there were significant differences in the alleles of IL-4-33, IL-4-590, and IL-4-1098 (p<**0.05**). The allele IL-4-590/C had the highest susceptibility (P<**0.001**, OR = 4.619, CI = 1.640-7.985), implying that people with this allele were 3.6 times more likely to develop COPD than those with the IL-4-33-590/T allele. COPD was associated with IL-4-33/C:C (P<**0.001**, OR = 29.5, CI = 11.138-57.647) and IL-4-1098/T:T (p<**0.001**, OR = 3.3, CI = 1.016-7.862) in susceptible patients. COPD (protective) was found to be negatively associated with IL-4-33/C:T (P = **0.009**, OR = 0.109, CI = 0.015-0.809), IL-4-590/C:T (P = **0.009**, OR = 0.397, CI = 0.195-0.810), IL-4-1098/G:G (P=**0.001**, OR = 0.133, CI = 0.054-0.331), and IL-4-1098/G:T (p<**0.001**,OR=0.209,CI=0.104-0.527). Negative relationship was observed between COPD and haplotypes IL-4/TTC (P<**0.001**, OR = 0.061, CI=0.008-0.443) and IL-4/GCC (P<**0.001**, OR = 0.193, CI = 0.076-0.488) . Only healthy subjects had IL-4/GCT, IL-4/GTC, and IL-4/GTT. In Hebei Province, polymorphisms in the IL-4 gene (loci 1098, -590, and -33) were found to be strongly linked to COPD (Chi *et al.*,2018)

Even though basophils make up less than 1% of lung-infiltrating cells, Shibata *et al.* discovered a previously unknown role for them in the initiation phase of emphysema formation in an elastase-induced murine model of COPD. After intranasal elastase

instillation, monocytes were recruited to the lungs and differentiated into interstitial macrophages (IMs), but rarely alveolar ones (AMs). In contrast to popular belief, IMs rather than AMs expressed high levels of matrix metalloproteinase-12 (MMP-12), which contributes to emphysema formation. Experiments on genetically engineered mice revealed that basophil-derived IL-4, a Th2 cytokine, acted on lung-infiltrating monocytes to promote their differentiation into MMP-12–producing IMs, causing alveolar wall destruction and emphysema development. Mice lacking IL-4 only in basophils could not have pathogenic MMP-12–producing IMs and thus develop emphysema. As a result, the basophil-derived IL-4/monocyte-derived IM/MMP-12 axis plays an essential role in emphysema formation and may be a potential target for slowing emphysema progression during the early stages of COPD (Shibata *et al.*,2018)

Flora et al. hypothesized that elastin-derived peptides (EP) might modulate the pattern of T lymphocyte cytokine expression in COPD patients. $CD4^+$ and $CD8^+$ T-cells were sorted from COPD patients' (n=29) and controls' (n=13) peripheral blood mononuclear cells (PBMC) and cultured with or without EP. Multicolour flow cytometry was used to examine cytokine expression in T-cell phenotypes, while desmosine concentration, a specific marker of elastin degradation, was measured in serum. COPD patients had a lower percentage of IL-4 (Th2) producing $CD4^+$ T-cells (35.33.4% and 26.32.4%, respectively, $p<$**0.005**) than controls, but no significant differences were found with IFN^- (Th1) or IL-17A. (Th17). Based on the percentage of IL-4 (Th2) generating CD4+ T-cells in COPD patients, two subpopulations were identified, with only one expressing high IL-4 levels associated with high levels of desmosine and strong smoking exposure (n=7). The percentage of $CD4^+$ T cells expressing IL-4 increased significantly in COPD patients ($p<$**0.005**) after stimulation with VGVAPG, a bioactive EP motif, but not in controls. In the presence of analogous peptides that block VGVAPG/elastin receptor (S-gal) interactions, the VGVAPG-induced increase in

IL-4 was inhibited. According to this study, the VGVAPG elastin peptide modulates CD4+ T-cell IL-4 production in COPD. Monitoring IL-4 in circulating $CD4^+$ T-cells may help characterize COPD phenotypes better and may open a new pharmacologic opportunity for COPD patients by stimulating CD4+ T-cells via the VGVAPG/S-gal receptor to favour an anti-inflammatory response (Flora *et al.*,2021).

COPD is characterized by inflammation. COPD is a disease with a well-documented inflammatory component that has been discussed in numerous studies. However, given the transversal nature of most of the studies conducted thus far and the potential for the misunderstanding regarding several external factors related to the lifestyle associated with levels of inflammatory biomarkers, which are, in turn, generally nonspecific, it is unclear whether these proteins are simply markers of the inflammatory process that accompanies COPD or if they play a significant role in the pathogenesis of the disease. Many studies have found a strong association between IL4 and its polymorphism and asthma, but no extensive data is available to find the link between *IL4RAQ576R* polymorphism and COPD due to the complex nature of disease.

OBJECTIVES OF STUDY

Chapter 3

Objectives of the study

The present piece of work is an attempt to study the following aspects that might be associated towards the associative risk for Chronic Obstructive Pulmonary Disease.

1. To investigate the role of *GSTT1* and *GSTM1* polymorphisms as a genetic modifier for risk towards COPD and its correlation with different clinical features within North Indian population.

2. To assess the role of *IL-4R(α)* polymorphism as a genetic modifier for risk towards COPD and its association with different clinical parameters within North Indian population.

MATERIAL AND METHODS

Chapter 4

Material and method

4.1 Sample collection

A cross-sectional study was conducted. The university's ethical committee approved the study protocol. Four hundred people were enrolled in the study, including 200 COPD cases and 200 controls from the Chest and TB hospital at the Government Medical College in Patiala. Participants in the study were requested to sign consent forms. A detailed questionnaire was filled out for each case and control cohort. The questionnaire asked for demographic and smoking history information. The patients' history, examination, and spirometry are used to diagnose COPD using the clinical criteria outlined in the GOLD guidelines (GOLD,2019). Medical data from cases were obtained, including bronchogenic cancer, biomass exposure, duration of COPD, Gold Severity, Modified Medical Research Council (mMRC), dyspnea scale, COPD Assessment Test (CAT) score, and other symptoms. Blood samples from healthy people were collected in EDTA-coated vacutainer tubes and stored at -20^{o}C until DNA extraction.

4.2Extraction of DNA from blood

Genomic DNA was extracted from blood samples of COPD patients and healthy individuals using standard Protein K digestion, phenol/chloroform extraction, and precipitation by ethanol (Barlett & White's method,2003).

4.2.1*Requirements*

- Washing Buffer
- Lysis Buffer
- Phenol: Chloroform: Isoamyl alcohol (P: C: I: 25:24:1)
- Chloroform: Isoamyl alcohol (C: I: 24:1)

- Isopropanol
- 70% Ethanol
- Tris-EDTA Buffer

4.2.2 *Preparation of Buffers*

Table 4.1: Preparation of washing buffer

Name of Component	Stock Concentration	Working Concentration
Sucrose	1 M	320mM
Triton X -100	100%	1%
$MgCl_2$	100mM	5mM
Tris Cl (pH=8)	100 mM	10mM

Table 4.2: Preparation of Lysis buffer

Name of Component	Stock Concentration	Working Concentration
Tris Cl (pH=8)	1M	400mM
SDS	10%	1%
EDTA	0.5M	60mM
NaCl	5M	150mM
Proteinase K	10mg/ml	100µg/ml

4.2.3 *The procedure of DNA extraction from peripheral blood*

1. 4 mL of blood was drawn, and an equal amount of washing buffer was added and thoroughly mixed.
2. It was subjected to centrifugation at 3500 rpm for 5 minutes. The supernatant was removed, and the pellet was resuspended in the buffer and centrifuged again. This procedure was carried out thrice.
3. The pellet was dissolved in 4 ml of lysis buffer and incubated at 46^{o}C overnight or 55^{o}C for 3-4 hours.
4. P: C: I (Phenol: Chloroform: Isoamyl alcohol) (25:24:1) was added in an equal volume of 4 ml and centrifuged at 8000 rpm for 10 minutes. This procedure was executed twice.
5. The upper aqueous layer was collected, and an equal volume of C: I (Chloroform: Isoamyl alcohol) (24:1) was added to the aqueous layer, then centrifuged at 6500 rpm for 5 minutes.
6. 2.5 times the volume of chilled absolute ethanol or the equivalent volume of chilled isopropanol was added to the aqueous layer.
7. For 1-2 hours, the solution was frozen at -20°C. It was centrifuged for 10 minutes at 12000 rpm at 4^{o}C.
8. The supernatant was discarded, and the DNA pellet was washed twice in chilled 70% ethanol for 5 minutes at 12000 rpm.
9. The pellet was air-dried, and ethanol was decanted. This air-dried pellet was dissolved in 50µl-150 µl of TE (Tris-EDTA) buffer and reserved for future

4.3 Qualitative analysis of DNA

4.3.1 *Requirements*

- Electrophoresis buffer (TBE or TAE)
- Ethidium bromide solution
- Electrophoresis - grade agarose
- Loading dye
- DNA ladder
- Horizontal gel electrophoresis apparatus
- Gel casting platform
- Gel combs
- Power supply

4.3.2*Procedure*

Table 4.3: Preparation of 5X TBE (Tris Borate EDTA) (1 liter / 1000ml)

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

The final volume was made up of water.

Table 4.4: Preparation of loading dye

Name of the component	100ml	50 ml
30% Sucrose	303.2.3g	15g
0.1% Bromophenol Blue	0.1g	0.05g
20mM EDTA	4 ml	2ml

4.3.3 *Preparation of agarose gel*

1. A 0.8 % agarose gel was prepared and melted (0.8g in 100 ml of 0.5X TBE).
2. Before casting on the gel platform, the agarose was cooled to 55^{o} C, and three µl of ethidium bromide was added.

3. The casting tray was set with combs, and melted agarose with a thickness of about 0.5-1 cm was poured.
4. It was ascertained that no bubbles were trapped beneath the combs, and bubbles were removed prior to gel setting.

4.3.4 *Loading and running gel*

1. The gel combs were carefully removed once the gel had solidified.
2. 300 ml of electrophoresis buffer was prepared.
3. The gel casting platform was placed in the electrophoresis tank, and 300 ml TBE buffer was added until the tops of the well were submerged. It was ensured that there were no air pockets trapped within the wells.
4. 3µl of autoclaved water, 1µl of dye, and 2µl of the DNA sample were mixed with loading into the gel.
5. The samples were carefully loaded into wells using a micropipette to avoid cross-contamination.
6. A DNA ladder was also loaded to determine the DNA band size.
7. The electrodes were connected to the power source, and the switch was turned on.
8. Electrophoresis was performed at 70V and 25A until the dye migrated to the desired distance. The electricity was turned off, and the agarose gel was photographed using Gel Doc after being visualized under a UV transilluminator.

4.4Quantification of DNA

4.4.1 *Quantification of DNA by Nanodrop*

1. 1µl of deionized water was pipetted on to lower optical surface of the Nanodrop.
2. The nanodrop software was opened, and the nucleic acid module was selected.

3. One µl of TE was loaded for blank measurement, and the “blank” option was selected from the screen.
4. After the blank was set, one µl of DNA sample was packed, and the “measure” option was clicked.
5. The concentration and purity of the DNA sample were determined automatically.

4.4.2 *Quantification of DNA by UV spectrophotometer*

DNA concentration and purity can also be measured by UV spectrophotometer, the standard method for quantifying DNA. The absorbance of the sample was measured at two wavelengths, A260 nm and A280 nm. The ratio of A260/A280 was used to check the concentration of DNA. If the balance is ~1.8, the DNA sample is pure and free of RNA and protein contamination. A ratio of ~ 2.0 or >1.8 implies that the sample contains RNA contamination, whereas a ratio significantly less than 1.8 indicates that the selection includes protein contamination. Double standard DNA with an A260 nm of 1.0 has a 50 µg/ml concentration. The concentration of DNA in the solution was calculated using the following formula:

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

4.5Multiplex Polymerase Chain Reaction (PCR) Amplification of *GSTT1* and *GSTM1*

Multiplex PCR systems are widely used in biological and medical research because they enable the amplification of many DNA fragments by using multiple primers within the same PCR reaction. It reduces the number of responses required to test a sample for various targets. It saves time and money and makes multiplex systems worthwhile, particularly when screening large numbers of samples (Sint *et al.*,2012). The primers'

relative concentration, the PCR buffer's concentration, the balance of magnesium chloride and deoxynucleotide concentrations, cycling temperatures, and the amount of template DNA and Taq DNA polymerase are all crucial to a successful multiplex PCR assay. An optimal combination of annealing temperature and buffer concentration is required in multiplex PCR to get a highly specific amplicon. Magnesium chloride concentration must be proportional to the amount of dNTP, and primer concentration should also be adjusted for each target sequence (Markoulatos *et al.*,2002).

4.5.1 *Requirements*

- PCR Buffer
- BSA (Bovine Serum Albumin)
- Forward primer
- Reverse primer
- dNTPs
- Taq DNA polymerase
- Deionized water
- DNA sample

4.5.2*Primer Design Parameters for Multiplex PCR:*

1. Primer Length

   Multiplex PCR assays require the design of many primers, so the designed primer must be of appropriate length. Primers with a length of 18-22 bases are frequently used.

2. Melting Temperature

   Primers with similar Tm are used, preferably between 55°C to 60°C. Primers with a higher Tm (preferably 75°C-80°C) are strongly suggested for sequences with a high GC content. For primers used in a pool, a Tm variation of 3°-5°C is acceptable.

3. Specificity

   When preparing a multiplex assay, it is critical to consider the specificity of designed primers to the target sequences, primarily because competition exists when multiple target sequences are in a single reaction vessel.

4. Avoid Primer Dimer formation

   With all of the primers present in the reaction medium, the designed primers must be tested for the formation of primer dimers. Dimerization results in amplification that is not specific.

4.5.3 *Primers used in Multiplex PCR*

***GSTM1* primers:**

Forward Primer: 5'-GAA CTC CCT GAA AAG CTA AAG C-3'

Reverse Primer: 5'-GTT GGG CTC AAA TAT ACG GTG G-3'

Band Size: 215bp

***GSTT1* primers:**

Forward Primer:5'-TTC CTT ACT GGT CCT CAC ATC TC-3'

Reverse Primer: 5'-TCA CCG GAT CAT GGC CAG CA-3'

Band size: 480bp

Albumin primers:

Forward primer: 5'-GCC CTC TGC TAA CAA GTC CTA C-3'

Reverse primer: 5'-CCC TAA AAA GAA AAT CGC CAA TC-3'

Band Size: 312bp

Table 4.5: A reaction mixture of multiplex PCR carried out for *GSTM1* and *GSTT1*

Reagents	Stock Concentration	Working Concentration	Amount for 1 reaction	Amount for 10 reactions +10%
Taq / PCR Buffer	10X	1X	2µl	22 µl
BSA	100X	10X	2µl	22µl
Forward Primer (GSTM1)	10 µM	0.5 µM	1 µl	11 µl
Reverse Primer (GSTM1)	10µM	0.5µM	1µl	11µl
Forward Primer (GSTT1)	10 µM	0.5 µM	1 µl	11 µl
Reverse Primer (GSTT1)	10µM	0.5µM	1µl	11µl
Forward Primer (Albumin)	10 µM	0.3 µM	0.6 µl	6.6 µl
Reverse Primer (Albumin)	10µM	0.3µM	0.6µl	6.6µl
dNTPs	10mM	0.2 mM	0.4 µl	4.4 µl
Taq Polymerase	5U/µl	3U/µl	0.6µl	6.6µl
DNA	-	-	1 µl	1 µl
H2O	-	-	8.8µl	96.8µl
Total	-	-	20 µl	210 µl

Table 4.6: Multiplex PCR conditions for *GSTM1* and *GSTT1*

Steps	Temperature	Time
1. **Initial Denaturation**	95°C	5 min
2. **Denaturation**	94°C	1 min
3. **Annealing**	59°C	1 min
4. **Extension**	72°C	1 min
5. **Final Extension**	72°C	5 min

4.5.4 *Procedure*

1. All the reagents were thawed.
2. The master mix was prepared in the following order: water, BSA, buffer, primers, dNTPs, and Taq polymerase.
3. The master mix was divided into respective PCR tubes and followed by adding the individual DNA template.
4. The content in PCR tubes was gently mixed by tapping it and was put in the thermal cycler.
5. After the reaction cycles were completed, the PCR samples were subjected to 2% agarose gel electrophoresis.
6. The results were visualized under a UV transilluminator.

4.6 *IL4RAQ576R* genotyping by PCR-RFLP

The genotype of IL4RAQ576R was determined by using the PCR-RFLP method according to the protocol of Oyama *et al*.,1994. The amplicon produced by PCR was 123bp in size which was amplified using the following primer sequences 5-GCCTTGTAACCAGCCTCTCCT-3 (Forward Primer) and 5-GCCCCCACCAGTGGCTACC-3 (Reverse Primer). The reaction mixture and PCR conditions are as follows:

Table 4.7: A reaction mixture of PCR carried out for *IL4RAQ576R*polymorphism

Reagents	**Stock Concentration**	**Working Concentration**	**Amount for 1 reaction**	**Amount for 10 reactions +10%**
Taq Buffer	10X	1X	1.5µl	16.5µl
BSA	100X	10X	1.5µl	16.5µl
Forward Primer	10µM	0.5µM	0.75µl	8.25µl
Reverse Primer	10µM	0.5µM	0.75µl	8.25µl
dNTPs	10 mM	0.2 mM	0.3µl	3.3µl
Taq Polymerase	5U/µl	1.5 U/µl	0.3µl	3.3µl
DNA	-	-	0.5 µl	0.5µl
H_2O	-	-	9.4µl	103.4µl
Total	-	-	15µl	

Table 4.8: PCR conditions for *IL4RAQ576R* polymorphism

Steps	Temperature	Time
6. **Initial Denaturation**	95°C	5 min
7. **Denaturation**	94°C	1 min
8. **Annealing**	59°C	1 min
9. **Extension**	72°C	1 min
10. **Final Extension**	72°C	5 min

4.6.1 Restriction digestion of *IL4RAQ576R*

The restriction digestion of PCR product was done using Msp I enzyme, which was incubated at 37ºC. The digested PCR products were then separated on 6% PAGE (Poly acrylamide gel electrophoresis) gel, and digested bands were visualised by EtBr staining. The restricted digested pattern observed in 6% acrylamide gel was as follows:

Table 4.9: The restricted digested pattern of *IL4RAQ576R*

Genotype	Band Size
AA- Wild type	107 bp, 16bp
AG- Heterozygous	107 bp, 89 bp, 18 bp, 16 bp
GG- Mutant	89 bp, 18bp, 16 bp

Table 4.10: The reaction mixture of enzymatic digestion

Reagents	Stock Concentration	Working Concentration	The amount for one reaction
Buffer	10X	1X	2µl
***Msp I* enzyme**	10U/µl	2U/µl	0.2µl
PCR product	-	-	5µl
H_2O	-	-	12.8µl
Total	-	-	20 µl

4.6.2 Components of PAGE

- 30% acrylamide solution (29:1w/v; Acrylamide: Bisacrylamide)
- TEMED (Tetramethylethylenediamine)
- 10% Ammonium persulphate (freshly prepared solution)
- 5X TBE solution

Table 4.11: Preparation of 6% PAGE gel

Gel %	Name of the reagents				
6%	**30% acrylamide(ml)**	**H_2O (ml)**	**5X TBE (ml)**	**10% APS (µl)**	**TEMED (µl)**
	2.4 ml	**7.2 ml**	**2.4 ml**	**200µl**	**10µl**

4.7 Statistical Analysis

The $\chi 2$ test for categorical data and the Student t-test for continuous variables were 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 *IL4RAQ576R* gene polymorphism using the χ^2 test. The Pearson's χ^2 test was used to see if there was a statistically significant difference in allele and genotype frequencies between cases and controls. To evaluate the risk of COPD with *GST* polymorphisms and *IL4RAQ576R* polymorphism, logistic regression analysis with adjustment for potential parameters (age and pack-years of smoking as continuous variables; and gender as a nominal variable) was used to determine the adjusted odds ratios (ORs) and 95% confidence intervals (CIs).All p values were two-sided, and p-values < 0.05 were statistically significant. Medical version 9.3.6.0 (Medcalc Software, Ostend, Belgium) and SPSS Version 20.0 were used for the statistical study (Chicago, IL, USA).

RESULTS

Chapter 5

Results

5.1 Genomic DNA

The extracted DNA was subjected to 0.8% agarose gel and prepared in 0.5X TBE for qualitative estimation. The genomic DNA was visualized under a UV transilluminator with the help of ethidium bromide, which stains the DNA fragments, as represented in **figure 6.**

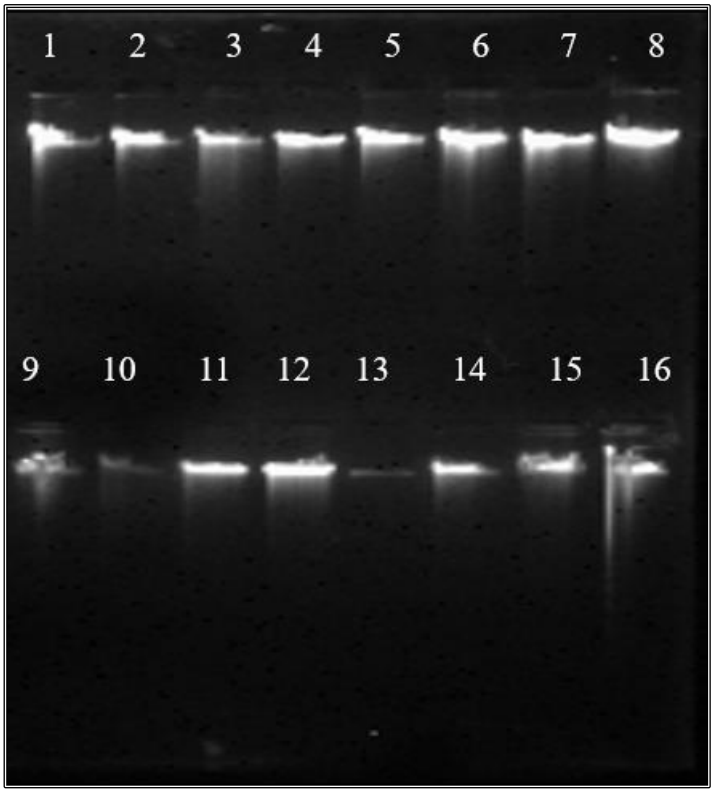

Figure 5.1: Pictorial depiction of 0.8% agarose gel for detecting genomic DNA isolated from peripheral blood. Lane 1- 16 contains genomic DNA of COPD cases and controls

5.2 Multiplex PCR amplification of *GSTM1* and *GSTT1*

Using thermal cycling parameters, appropriate primer sets amplified the GSTM1, GSTT1, and albumin genes. The amplicon was separated using ethidium bromide as a staining

agent on a 1.5% agarose gel. The visible and distinct DNA bands indicated that the primer combinations were adequate for all genes. The figure shows the PCR amplified DNA products obtained with *GSTM1, GSTT1*, and Albumin-specific primer pairs. The DNA bands for *GSTM1, GSTT1*, and Albumin were 215 bp, 480 bp, and 312 bp, respectively. Albumin acted as an internal control **(Figure 5.2).**

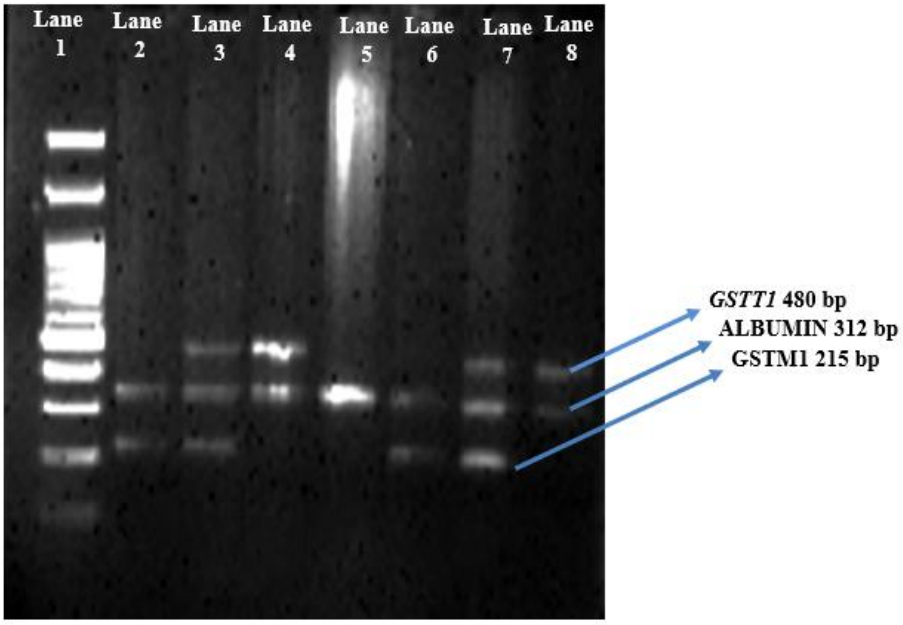

Figure 5.2:2% Agarose gel of PCR products for the detection of GST polymorphism. Lane 1: Marker (M) (100 bp); Lane 2,6 :GSTM1(+), Albumin; Lane 3,7: GSTM1(+), Albumin, GSTT1(+); Lane4,8: Albumin, GSTT1(+); Lane 5: GSTM1(-), Albumin, GSTT1(-)

5.3 Restriction fragment length polymorphism of *IL4RAQ576R* gene

The digested PCR product of *IL4RAQ576R* gene polymorphism with *Msp I* resulted in one fragment of 107bp in case of wild genotype two fragments of 107 bp, 89bp in case of heterozygous genotype and one fragment of 89 bp in case of mutant genotype when subjected to 6% PAGE gel and stained by EtBr (**Figure 5.3**).

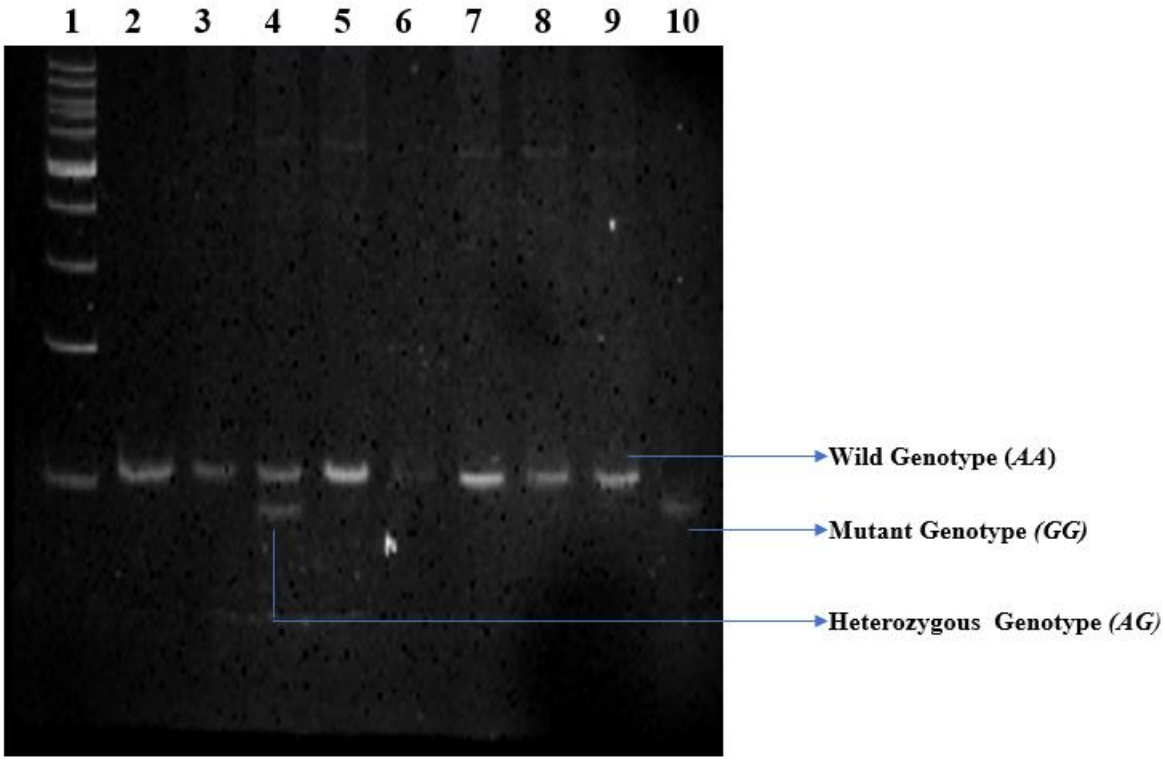

Figure 5.3: 6% PAGE gel of digested PCR products for the detection of genotypes of IL4RAQ576R polymorphism. Lane1: Marker (M) (100bp); Lane 2: Uncut/ PCR product(123bp); Lane 3,5,6,7,8,9: Wild (*AA*); Lane 4: Heterozygous genotype (*AG*); Lane 10: Mutant Genotype (*GG*)

5.4 Characteristics of subjects

The distribution of demographic characteristics of COPD cases and controls includes age, gender, smoking status, pack-years, smoking index, GOLD severity, mMRC, CAT score, GOLD "ABCD," duration of COPD, as shown in **table 5.1**. A case-control study consisted of 200 COPD cases and 200 healthy individuals. The mean age of patients was 58±11.96 (Range 21-80), whereas the mean age of controls was 43.46±15.84 (Range 9-84), and the distribution was significant (**p<0.0001**). The study comprised 183 (91.5%) males and 28 (14%) females in the case group and 115 (57.5%) males and 85 (42.5%) females in the control group. There was a significant distribution of males and females in controls and patients(**p<0.0001**).

Table 5.1: Distribution of demographic characteristics of COPD cases and controls

Parameters	Cases, (n%) N=200	Controls, (n%) N=200	p value
Age (Years)			**<0.0001**
Mean ± SD	58 ± 11.96	43.46 ± 15.84	
Range	(21-80)	(9-84)	
Gender			**<0.0001**
Male	183(91.5)	115(57.5)	
Females	17(8.5)	85(42.5)	
Smoking Status			**<0.0001**
Smokers	172(86)	24(12)	
Non-Smokers	28(14)	176(88)	
Pack Years			**0.0001**
Mean ± SD	44.83 ± 39.58	12.69 ± 13.19	
Smoking Index			**0.0002**
<400	96(48)	23(11.5)	
≥400	76(38)	1(0.5)	
GOLD Severity			
I	7(3.5)		
II	71(35.5)		
III	79(39.5)		
IV	43(21.5)		
mMRC			
mMRC< 2	32(16)		
mMRC ≥ 2	168(84)		
CAT score			
CAT < 10	32(16)		
CAT ≥ 10	168(84)		
GOLD "ABCD"			
A	29(14.5)		
B	146(73)		
C	3(1.5)		
D	22(11)		
Duration of COPD			
< 2 years	78(39)		
2-5 years	56(28)		
5-10 years	28(14)		
>10 years	38(19)		

SD=Standard Deviation. p values were derived from the Pearson Chi-square test except for age and packyears; the Student t-test was used for age and pack years. All p values are two-sided **p<0.005** was considered statistically significant.

In the present study, there were 172 (86%) smokers and 28 (14%) non-smokers in cases and 24 (12%) smokers and 176 (88%) non-smokers in controls. Smoking was a significant risk factor for COPD (**p<0.0001**). The mean pack-years among COPD patients was 44.83 ±39.58, significantly higher than healthy subjects (**p=0.0001**) who had mean packyears of 12.69±13.19. The smoking index < 400 was found in 96(48%) cases, and 23 (11.5%) controls, a smoking index ≥ 400 was observed in 76 (38%) cases, and 1 (0.5%) control and its distribution among patients and healthy individuals was significant (**p=0.0002**). The GOLD severity based on spirometry values was divided into four groups: I, II, III & IV. Group I comprised of 7 (3.5%) individuals, Group II had 71 (35.5%) individuals, Group III consisted of 79 (39.5%) individuals, and Group IV had 43 (21.5%) individuals with COPD cases. In contrast, no such group was present in healthy subjects. mMRC grading was classified into two groups according to GOLD guidelines, i.e., mMRC<2 and mMRC≥2. mMRC<2 had 32 (16%) cases, and mMRC≥2 had 168 (84%) cases of COPD, whereas no such grading is applicable in healthy controls. The CAT score is a diagnostic tool other than mMRC grades to assess COPD severity based on symptoms besides dyspnea. CAT score was categorized into two groups recommended by GOLD guidelines, i.e., CAT <10 and CAT≥10. The CAT <10 was observed in 32(16%) cases, and CAT≥10 was observed in 168 (84%) cases of COPD. GOLD ABCD was another essential parameter among COPD cases. It included four groups, namely Group A, Group B, Group C, and Group D. 29 (14.5%) individuals belonged to Group A, 146 (73%) belonged to Group B, 3(1.5%) belonged to C and 22 (11%) belonged to Group D among the cases. The duration of COPD was also characterized among COPD subjects and was divided into four groups, i.e., <2 years,2-5 years, 5-10 years, and >10 years.The majority of our cases had COPD for less than two years, which comprised 78 (39%) patients, 56 (28%) patients who had COPD for 2-5 years, 28(14%) patients who had this disease for 5-10 years, and 38 (19%) patients had COPD for more than ten years.

5.4.1 Characteristics of COPD cases based on GOLD severity

The classification of airflow limitation severity in COPD based on post-bronchodilator FEV_1 is GOLD I, GOLD II, GOLD III, and GOLD IV. The pie chart **(Figure 5.4)** illustrates that 7(3.50%) patients had GOLD I severity which is mild and has FEV1 ≥80% predicted, and 71(35.50%) COPD cases had GOLD II severity which is moderate and has 50% ≤ FEV1 < 80% predicted,79(39.50%) subjects had GOLD III severity which is severe and has 30% ≤ FEV1 < 50% indicated and 43(21.50%) patients had GOLD IV severity which is very strict and has FEV1 <30% predicted.

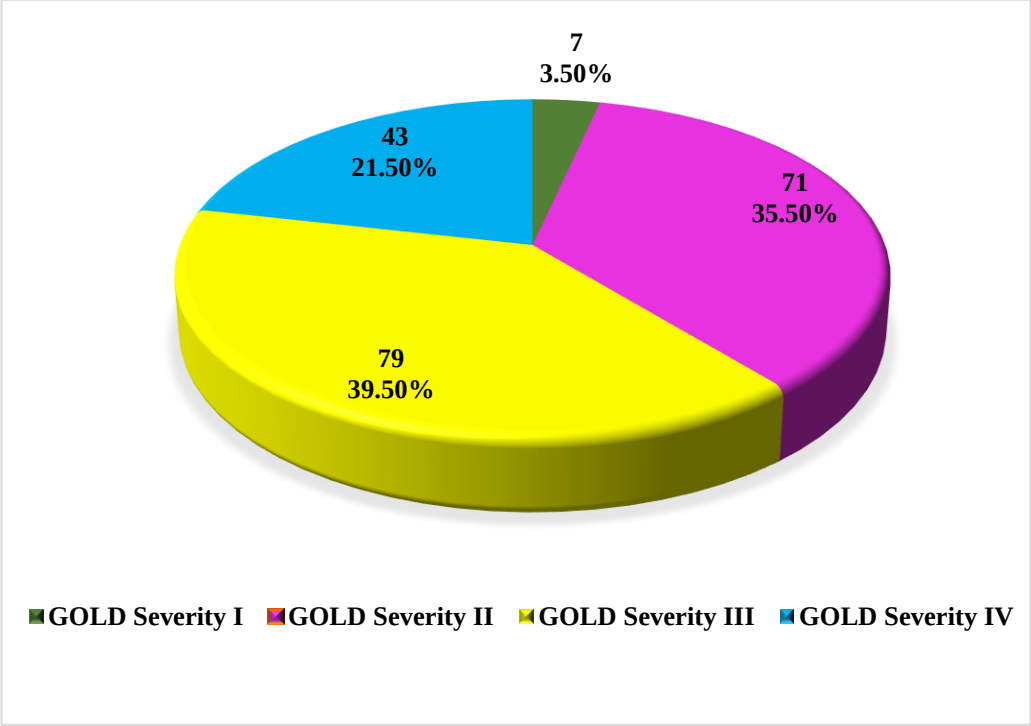

Figure 5.4: Pie chart illustration of GOLD severity among COPD cases.

5.4.2 Characteristics of COPD cases based on GOLD ABCD symptoms

GOLD ABCD is the refined assessment tool for symptoms or risk of exacerbations of COPD. It is classified as GOLD A, GOLD B, GOLD C and GOLD D. GOLD A comprised 29 (14%) patients with few symptoms and a low risk of exacerbations; GOLD B formed 146 (73%) COPD subjects who had more symptoms and low risk of exacerbations. GOLD C consists of 3 (2%) patients who had fewer symptoms but a higher risk of exacerbations, and GOLD D includes 22 (11%) cases who had more symptoms and increased risk of exacerbations. as represented in the pie chart **(Figure 5.5)**

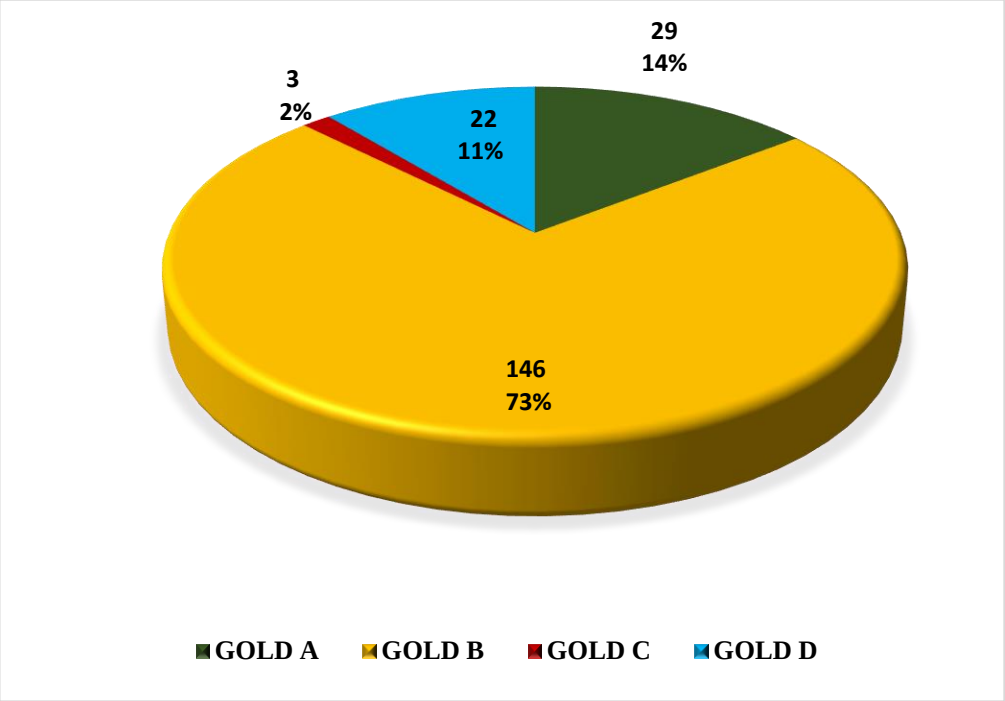

Figure 5.5: Pie chart illustration of the distribution of GOLD ABCD among COPD patients.

5.4.3 Characteristics of COPD cases based on mMRC grade

mMRC (Modified Medical Research Council) scale is used to assess the severity of breathlessness (dyspnea) in respiratory disorders, especially COPD. As per the GOLD guidelines, mMRC was categorized into two groups, i.e., mMRC<2 and mMRC≥2. In our investigation, 32(16%) COPD cases had mMRC<2, and 168 (84%) COPD patients had mMRC≥2 indicating a large number of COPD subjects had severe dyspnea, as shown in **Figure 5.6.**

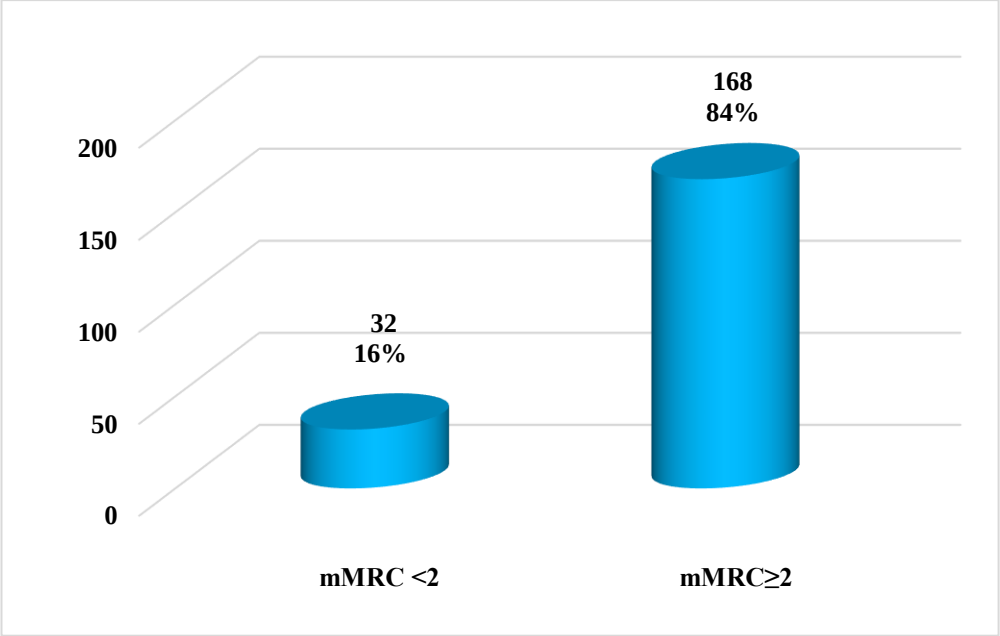

Figure 5.6: Graphical representation of the distribution of the mMRC scale in COPD subjects.

5.4.4 Characteristics of COPD cases based on CAT score

CAT (COPD Assessment Test) is a multidimensional method that evaluates not only dyspnea but also other symptoms and health status of COPD subjects. As per the GOLD guidelines, the CAT score was categorized into two groups, i.e., CAT<10 and CAT≥10. In our investigation, 32(16%) COPD cases had CAT<10, and 168 (84%) COPD patients had CAT≥10 indicating a large number of COPD subjects had severe dyspnea, as shown in **Figure 5.7.**

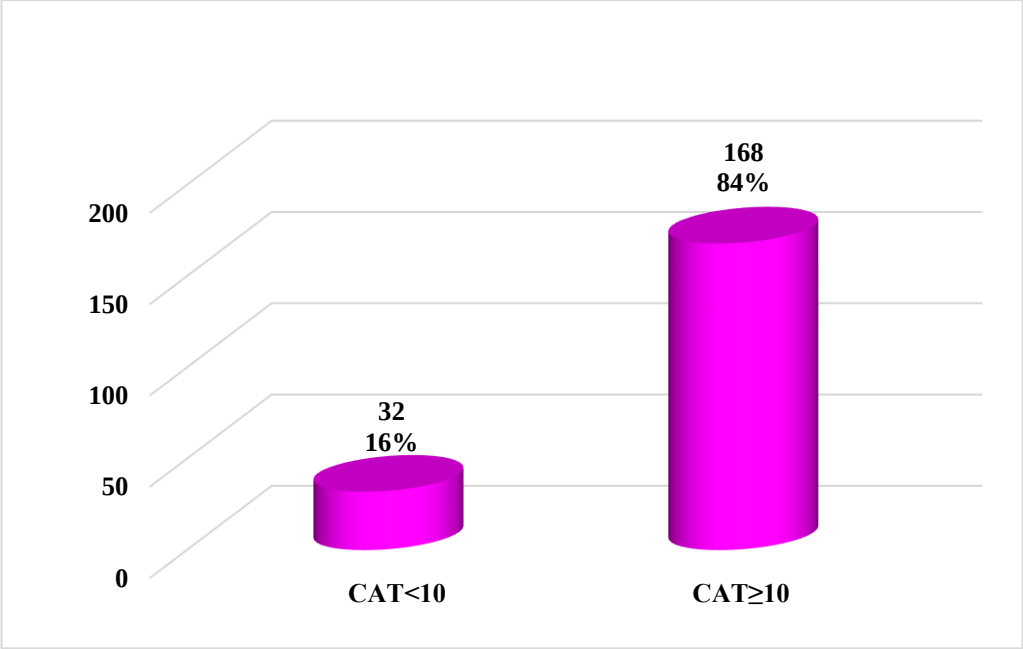

Figure 5.7: Graphical representation of the distribution of CAT scores in COPD subjects.

5.4.5 Characteristics of COPD cases based on the duration of COPD

The duration of COPD was also characterized among COPD cases and was divided into four groups, i.e., <2 years,2-5 years, 5-10 years, and >10 years.The majority of our cases had COPD for less than two years, which comprised 78 (39%) patients, 56 (28%) patients who had COPD for 2-5 years, 28(14%) patients who had this disease for 5-10 years, and 38 (19%) patients had COPD for more than ten years **(Figure 5.8)**

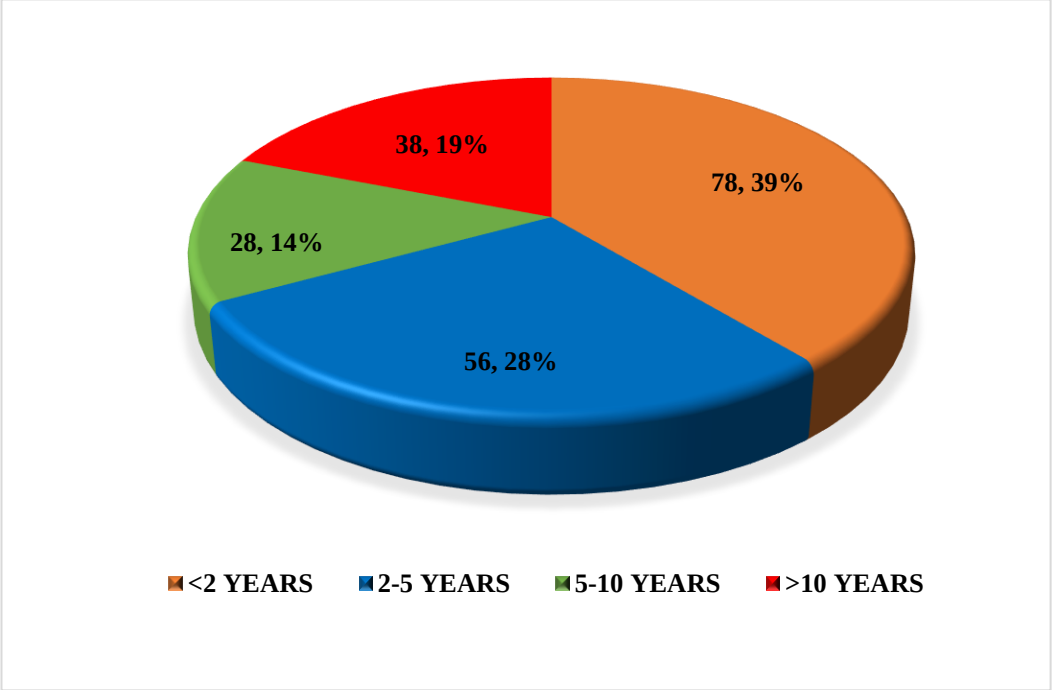

Figure 5.8: Graphical illustration of the distribution of COPD patients based on COPD duration.

5.5 *Results of GSTs polymorphism with COPD and its clinical parameters*

5.5.1 Distribution and association of GST polymorphism with COPD risk

The relationship of GST polymorphism with COPD was evaluated in our research. The *GSTT1*(-) null genotype was 34.5% in COPD cases and 20.5% in healthy subjects. A strong statistical association between *GSTT1*(-) null genotype and COPD risk was observed (OR= 2.04, **p=0.0019**, 95%CI=1.30–3.20). When adjusted with covariates such as age, sex, and smoking status, a significant association was observed in the null genotype of *GSTT1(-)* and COPD risk (AOR =2.90, **p=0.003**, 95%CI=1.43-5.87). The *GSTM1(-)* null genotype was present in 36.5 % of cases and was similar to the controls with 36.5% of *GSTM1(-)* deletion. No association between the GSTM1(-) genotype and COPD risk was found. (OR = 1.0, p =1, 95% CI = 0.66–1.50) (**Table 5.2**).

Table 5.2: Distribution and association of GST polymorphism with COPD risk

	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	127(63.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	73(36.5)	1.0	1	0.98 (0.52-1.84)	0.96
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	131(65.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	69(34.5)	2.04 (1.30-3.20)	**0.0019**	2.90 (1.43-5.87)	**0.003**

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 Combinatorial association of GST polymorphism towards susceptibility of COPD

Our study evaluated the relationship between COPD risk and different genotypic combinations. The *GST M1+/T1+* genotype was found in 75 (37.5%) subjects of the COPD cases and 96 (48%) controls. The *GST M1+/T1-* genotype was reported in 52 (26%) COPD individuals and 31 (15.5%) healthy individuals. A significant association was observed

between *GST M1+/T1-* genotype and COPD susceptibility with a two-fold increase in risk (OR=2.14, **p**=**0.005**, 95% CI=1.25-2.67), and after adjusting with different covariates such as age, sex, and smoking status, the risk increased by 2.9 folds (AOR=2.93, **p**=**0.009**, 95% CI=1.29-6.65). *GST M1-/T1-* was present in 17(8.5%) COPD patients and 10 (5%) healthy patients. Despite a two-fold increase in risk (OR=2.17, P= 0.06, CI=0.94-5.02), no association was found between *GST M1-/T1-* genotype and COPD. The *GST M1-/T1+* genotype was present in 56 (28%) COPD patients and 63 (31.5%) healthy patients. There was no correlation between COPD risk and *GST M1-/T1+* genotype. (OR=1.13, p=0.59, 95%CI=0.71-18.2; AOR=1.22, p=0.60, 95%CI=0.57-2.61) (**Table 5.3**)

Table 5.3: Combinatorial association of GST polymorphism towards susceptibility of COPD

	Controls	**Cases**				
Genotype	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GST M1+/T1+	96(48)	75(37.5)	1.0 (Reference)	-	1.0 (Reference)	-
GST M1-/T1-	10(5)	17(8.5)	2.17(0.94-5.02)	0.06	2.66(0.79-8.94)	0.11
GST M1+/T1-	31(15.5)	52(26)	2.14(1.25-3.67)	**0.005**	2.93(1.29-6.65)	**0.009**
GST M1-/T1+	63(31.5)	56(28)	1.13(0.71-1.82)	0.59	1.22(0.57-2.61)	0.60

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 GST polymorphism and COPD based on gender

Our study comprised 298(74.5%) males and 102(25.5%) females. In the category of males, the occurrence of *GSTT1(-)* null genotype was 31% (62) in cases and 13% (26) in controls. The COPD risk was significantly associated with *GSTT1*(-) null deletion among males, (OR =1.75, **p**=**0.03**,95% CI= 1.02-2.99) However, *GSTM1(-)* null genotype was 33.5% (67) in COPD subjects and 21% (42) in healthy subjects and no association was observed between *GSTM1(-)* null genotype and COPD risk among males (OR=1.00, p=0.9, 95%CI =0.61-1.62) (**Table 5.4**)In females, the presence of the *GSTT1(-)* null genotype was 7(3.5%) in cases and

15(7.5%) in healthy individuals. A strong correlation was found between GSTT1(-) null genotype and COPD risk among females (OR=3.26, p=0.03, 95% CI= 1.07-9.96).

A seven-fold increase in COPD susceptibility in females who had *GSTT1(-)* null genotype was observed after adjusting it with factors like age and smoking status (AOR=7.11, **p**=**0.003**,95% CI = 1.90-26.64) (**Table 6**). However, the *GSTM1*(-) null genotype was present in 3% (6) COPD patients and 15.5% (31) in controls among females. No correlation was found between *GSTM1*(-) null genotype and COPD susceptibility (OR =0.95, p= 0.92, 95%CI= 0.32-2.82) (**Table 5.4).**

Table 5.4: Association of GST polymorphism and COPD based on gender

Male						
	Controls	**Cases**				
Genotype GSTM1	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	73(36.5)	116(58)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	42(21)	67(33.5)	1.00(0.61-1.62)	0.98	0.91(0.433-1.88)	0.79
Genotype GSTT1	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	89(44.5)	121(60.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	26(13)	62(31)	1.75(1.02-2.99)	**0.03**	2.04 (0.91-4.59)	0.08
Female						
	Controls	**Cases**				
Genotype GSTM1	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	54(27)	11(5.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	31(15.5)	6(3)	0.95(0.32-2.82)	0.92	1.24(0.36-4.24)	0.72
Genotype GSTT1	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	70(35)	10(5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	15(7.5)	7(3.5)	3.26(1.07-9.96)	**0.03**	7.11(1.90-26.64)	**0.003**

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 and smoking. A **p<0.05** was considered to be statistically significant

5.5.4 Association of GST polymorphism and mMRC for COPD risk

We evaluated the relationship of mMRC with GST polymorphism in COPD patients and healthy subjects. Two groups were categorized based on the cut point of mMRC<2 and mMRC ≥ two as recommended by GOLD 2011 guidelines. In the first group, i.e., mMRC<2,

GSTT1 (-) null genotype was observed in 12 cases (6%), and 41(20.5%) controls. There was a significant association of *GSTT1* (-) null genotype with mMRC in COPD patients (OR=2.32, p=**0.03**, 95%CI = 1.05- 5.14). On the contrary, *GSTM1(-)* null genotype was present in 17(8.5%) cases and 73(36.5%) controls. No significant relationship was found between *GSTM1*(-) and mMRC in COPD cases (OR=1.97, p =0.07, 95%CI=0.92 - 4.18) (**Table 7**). Individuals having mMRC ≥2, GSTT*1* (-) null genotype was reported in 57(28.5%) COPD patients and 41(20.5%) controls. A significant association was found between *GSTT1*(-) and

mMRC in COPD cases (OR=1.99, **p**=**0.004**, 95% CI=1.24,3.18). When adjusted with parameters like age, gender, and smoking status, it showed a strong correlation between *GSTT1*(-) null genotype with mMRC≥2 for COPD risk (AOR=4.13, **p**=**0.0008**, 95% CI=1.80-

9.46) However, *GSTM1*(-) was present in 56(28%) cases, and 73(36.5%) healthy subjects and no correlation was found between *GSTM1*(-) with mMRC≥2 for COPD risk (OR=0.86, p=0.52,95% CI=0.56-1.33) (**Table 5.5**).

Table 5.5: Association of GST polymorphism and mMRC for COPD risk.

mMRC (mMRC<2)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	15(7.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	17(8.5)	1.97 (0.92-4.18)	0.07	2.37 (0.96-5.84)	0.06
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	20(10)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	12(6)	2.32 (1.05-5.14)	**0.03**	1.61 (0.62-4.15)	0.32
mMRC (mMRC ≥2)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	112(56)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	56(28)	0.86 (0.56-1.33)	0.52	0.74 (0.36-1.50)	0.41
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	111(55.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	57(28.5)	1.99 (1.24-3.18)	**0.004**	4.13(1.80-9.46)	**0.0008**

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 Association of GST polymorphism and CAT score for COPD risk

The association between GST polymorphism and CAT score was evaluated in our study. Individuals were grouped in two categories suggested by GOLD 2011 guidelines, i.e., CAT score<10 and CAT score≥10. In the first group of CAT scores <10, the presence of *GSTT1*(-) null genotype was in 6% (12) cases and 20.5% (41) controls. A significant two-fold increase in COPD risk was observed with the *GSTT1* (-) null genotype (OR=2.32, **p**=**0.03**, CI=1.05-5.14). On the other hand, *GSTM1*(-) genotype was observed in 17(8.5%) cases and 73(36.5%) healthy subjects. No significant relationship was found between *GSTM1*(-) with CAT score<10 in COPD cases (OR=1.97, p=0.07, 95%CI=0.92 - 4.18) (**Table 5.6**). In the second group, individuals having CAT score ≥10, *GSTT1* (-) null genotype was reported in 57(28.5%) COPD patients and 41(20.5%) controls. A significant association was observed between *GSTT1(-)* null genotype and CAT score among the COPD patients (OR=1.99, **p**=**0.004**, 95% CI=1.24,3.18). When adjusted with parameters like age, gender, and smoking status, a strong correlation between *GSTT1*(-) null genotype with CAT score ≥10 for COPD risk was observed (AOR=4.13, **p**=**0.0008**, 95% CI=1.80-9.46). However, *GSTM1*(-) was present in 56(28%) cases and 73(36.5%) healthy subjects and no correlation was found between *GSTM1*(-) with CAT score ≥10 for COPD risk (OR=0.86, p=0.52,95% CI=0.56-1.33) (**Table 5.6**).

Table 5.6: Association of GST polymorphism and CAT score for COPD risk

CAT (CAT<10)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	15(7.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	17(8.5)	1.97 (0.92-4.18)	0.07	2.37(0.96-5.84)	0.06
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	20(10)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	12(6)	2.32 (1.05-5.14)	**0.03**	1.61 (0.62-4.15)	0.32
CAT (CAT ≥10)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	112(56)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	56(28)	0.86 (0.56-1.33)	0.52	0.74 (0.36-1.50)	0.41
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	111(55.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	57(28.5)	1.99 (1.24-3.18)	**0.004**	4.13 (1.80-9.46)	**0.0008**

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 Association of GST polymorphism and GOLD severity for COPD risk

We have also assessed the relationship between GST polymorphism and GOLD severity. Subjects were divided into two groups: Group A included mild (FEV1≥ 80% predicted) and moderate (50% ≤FEV1 <80% indicated) cases of airflow obstruction, and Group B had severe (30% ≤FEV1 <50% predicted) and very intense (FEV1<30% indicated) cases of airflow limitation.

In Group A, *GSTT1*(-) null genotype was found to be 13% (26) in COPD individuals and 20.5% (41) in healthy individuals. *GSTT1*(-) genotype was found to have a significant relationship with GOLD severity for susceptibility to COPD (OR=1.93, **p=0.02**, 95%CI=1.08-3.47). However, no association was found between*GSTM1(-)* and GOLD severity of airway limitation in COPD subjects (**Table 5.7**).

In Group B, *GSTT1*(-) null genotype was found in 43 (21.5%) cases, and 41 (20.5%) controls with a two-fold risk of severity in COPD patients, and the association was found to be significant. (OR=2.11, **p**=**0.003**, 95% CI=1.27-3.50). After adjusting it with various covariates like age, sex, and smoking status, there was an associated risk between *GSTT1(-)* null individuals and GOLD severity in COPD patients. (AOR=3.33, **p**=**0.005**, 95%CI=1.42-7.81) However, no association was found between*GSTM1(-) and* GOLD severity of airway limitation in COPD subjects (**Table 5.7**)

Table 5.7: Association of GST polymorphism and GOLD severity for COPD risk

GOLD SEVERITY (Group A - Mild +Moderate)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	45(22.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	33(16.5)	1.27(0.74-2.17)	0.37	1.34(0.63-2.86)	0.44
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	52(26)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	26(13)	1.93(1.08-3.47)	**0.02**	1.93(0.84-4.47)	0.12
GOLD SEVERITY (Group B – Severe+ Very Severe)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	82(41)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	40(20)	0.84(0.52-1.36)	0.49	0.85(0.40-1.80)	0.67
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	79(39.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	43(21.5)	2.11(1.27-3.50)	**0.003**	3.33(1.42-7.81)	**0.005**

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 GST polymorphism and GOLD "ABCD" symptom-based assessment for COPD risk

GOLD ABCD is a refined evaluation tool that offers symptom burden and exacerbation risk information that can be used to guide treatment. According to GOLD 2011 guidelines, we divided our COPD cases into four groups designated A, B, C, and D. We evaluated the relationship between GST polymorphism and GOLD "ABCD" symptom-based assessment.

In category A, 5.5% (11) COPD cases and 20.5% (41) healthy subjects had *GSTT1(-)* null genotype. A significant correlation was observed between *GSTT1(-)* and GOLD A category among COPD individuals (OR=2.36, **p**=**0.04**, 95%CI=1.03-5.40). In category B, *GSTT1(-)* null genotype was present in 53 (26.5 %) cases and 41 (20.5%) controls. A significant association was found between *GSTT1 (-)* and GOLD B category with two-fold risk in patients harbouring *GSTT1 (-)* null genotype (OR=2.2, **p**=**0.001**, CI=1.36-3.57). A strong correlation of *GSTT1 (-)* with GOLD B category was observed after adjusting it with parameters like age, sex, and smoking status with a five-fold increase in COPD risk (AOR=5.06, **p**=**0.0003**, 95%CI=2.11-12.11). *GSTT1(-)* null genotype. On the other hand, *GSTT1(-)* showed no correlation with Group C and D among COPD individuals. Furthermore, *GSTM1(-)* null genotype and any of the groups as mentioned earlier showed no significant association **(Table 5.8**)

Table 5.8: Association of GST polymorphism and GOLD "ABCD" symptom-based assessment for COPD risk

GOLD (Gold A)						
	Controls	**Cases**				
Genotype ***GSTM1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	14(7)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	15(7.5)	1.86 (0.85-4.07)	0.11	2.26 (0.90-5.65)	0.08
Genotype ***GSTT1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	18(9)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	11(5.5)	2.36 (1.03-5.40)	**0.04**	1.63 (0.63-4.32)	0.30
GOLD (Gold B)						
	Controls	**Cases**				
Genotype ***GSTM1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	98(49)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	48(24)	0.85 (0.54-1.33)	0.48	0.80(0.39-1.66)	0.56
Genotype ***GSTT1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	93(46.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	53(26.5)	2.2 (1.36-3.57)	**0.001**	5.06 (2.11-12.11)	**0.0003**
GOLD (Gold C)						
	Controls	**Cases**				
Genotype ***GSTM1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	1(0.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	2(1)	3.4(0.31-39.03)	0.31	4.37 (0.33-57.39)	0.26
Genotype ***GSTT1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	2(1)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	1(0.5)	1.93 (0.17-21.91)	0.59	1.39 (0.10-18.53)	0.80
GOLD (Gold D)						
	Controls	**Cases**				
Genotype ***GSTM1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	14(7)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	8(4)	0.99 (0.39-2.48)	0.98	0.89 (0.25-3.10)	0.85
Genotype ***GSTT1***	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	18(9)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	4(2)	0.86 (0.27-2.68)	0.79	0.56 (0.13-2.44)	0.44

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

5.5.8 Correlation of GST polymorphism and COPD duration

We studied the association between GST polymorphism and COPD duration. COPD duration was classified into four categories, i.e., <2 years, 2-5 years, 5-10 years, and >10 years. In the < 2 years category, *GSTT1(-)* genotype was reported in 28(14%) cases and 41(20.5%) healthy participants. There was a significant association of *GSTT1(-)* and COPD duration (OR= 2.17, p=**0.008,** 95%CI=1.22-3.86). In the 2-5 years category, 8.5% (17) COPD patients and 20.5% (41) controls had *GSTT1(-)* genotype. After adjusting with covariates like age, gender, and smoking status, a significant relationship was observed between *GSTT1(-)* and COPD duration of 2-5 years (AOR=2.76, **p=0.04**,95% CI=1.03-7.33). In the >10 years category, *GSTT1(-)* genotype was present in 16 (8%) COPD cases and 41(20.5%). A significant correlation was observed between *GSTT1(-)* and COPD duration (OR=2.82**, p=0.005,** 95% CI=1.39-5.85**).** However, *GSTT1(-)* showed no correlation with the 5-10 years category among COPD individuals. Moreover, *GSTM1(-)* null genotype and any of the groups mentioned above showed no significant association **(Table 5.9)**

Table 5.9: Correlation of GST polymorphism and COPD duration

DURATION OF COPD (<2 years)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	49(24.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	29(14.5)	1.02(0.59-1.77)	0.91	1.17(0.53-2.58)	0.68
Genotype GSTT1	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	50(25)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	28(14)	2.17(1.22-3.86)	**0.008**	1.66 (0.71-3.85)	0.23
DURATION OF COPD (2-5 years)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	36(18)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	20(10)	0.96(0.52-1.79)	0.91	0.93(0.39-2.20)	0.87
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	39(19.5)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	17(8.5)	1.69(0.86-3.28)	0.12	2.76(1.03-7.33)	**0.04**
DURATION OF COPD (5-10 years)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	18(9)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	10(5)	0.96(0.42-2.20)	0.93	1.17(0.41-3.37)	0.75
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	20(10)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	8(4)	1.55(0.63-3.77)	0.33	2.11(0.62-7.18)	0.22
DURATION OF COPD (>10 years)						
	Controls	**Cases**				
Genotype *GSTM1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTM1+	127(63.5)	24(12)	1.00 (Reference)	-	1.00 (Reference)	-
GSTM1-	73(36.5)	14(7)	1.01(0.49-2.08)	0.96	1.02(0.34-3.08)	0.96
Genotype *GSTT1*	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GSTT1+	159(79.5)	22(11)	1.00 (Reference)	-	1.00 (Reference)	-
GSTT1-	41(20.5)	16(8)	2.82(1.39-5.85)	**0.005**	2.43(0.78-7.55)	0.12

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 IL4RAQ576R gene polymorphism with COPD and its clinical parameters*

5.6.1 Distribution and association of *IL4RAQ576R* polymorphism with COPD risk

The relationship of *IL4RAQ576R* polymorphism was evaluated in our investigation of COPD susceptibility. In the codominant model, heterozygous *(AG)* genotype was found in 34.15% of COPD patients and 33.49% of controls. No significant association was observed between the heterozygous (*AG*) genotype and COPD risk (OR=1.07, 95% CI=0.70-1.62, p=0.74) when compared to the wild-type (*AA*) genotype. Even when it was adjusted with covariates such as age, gender, and smoking status, no substantial relationship was found (AOR=1.06, p= 0.85,95% CI=0.57-1.96). The mutant (*GG*) genotype was observed in 3.96% of COPD patients and 1.47% of healthy individuals. Although the COPD risk was higher with this mutant genotype, roughly 3-fold, the association was not significant (OR=2.81, 95% CI=0.73-10.85, p=0.13). In the dominant model (*AA vs AG+GG*) and recessive model (*AA+AG vs GG*), these genotypes did not show any association for COPD susceptibility, as shown in **Table 5.10**.

Table 5.10: Overall distribution and association of *IL4RAQ576R* polymorphism with COPD risk

OVERALL						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	125 (61.88)	1.00 (Reference)	-	1.00 (Reference)	-
AG	68 (33.49)	69 (34.15)	1.07 (0.70-1.62)	0.74	1.06 (0.57-1.96)	0.85
GG	3 (1.47)	8 (3.96)	2.81 (0.73-10.85)	0.13	2.73 (0.37-19.95)	0.32
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	125 (61.88)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	71 (34.97)	77 (38.11)	1.14 (0.76-1.71)	0.51	1.12 (0.61-2.07)	0.69
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200(98.52)	194 (96.03)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	8 (3.96)	2.74 (0.71-10.51)	0.13	2.72 (0.41-18.10)	0.29

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 *IL4RAQ576R* polymorphism and CAT score for COPD risk

Our study evaluated the association between*IL4RAQ576R* polymorphism and CAT score. Individuals were grouped in two categories suggested by GOLD 2011 guidelines, i.e., CAT score<10 and CAT score≥10. In the first group of CAT scores <10, in the codominant model, the presence of heterozygous (*AG*) genotype was observed in 9.40% of cases and 33.49% in healthy individuals *(AA)*. A significant two-fold increase in COPD risk was honoured with the heterozygous (*AG*) genotype in comparison to the wild genotype (*AA*) (OR=2.83, 95% CI=1.32-6.08, p=**0.007**). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status (AOR=2.76, 95%CI=1.12-6.80, p=**0.02**). In the dominant model (*AA vs AG+GG*), the combined heterozygous and mutant genotype (*AG+GG*) was observed in 9.40% of COPD individuals and 34.97% of healthy individuals when compared to the wild genotype (*AA*). The combined association of *AG+GG* genotype for COPD susceptibility was strongly correlated (OR=2.71, 95%CI=1.26-5.82, p=**0.01**). When adjusted with parameters like age, gender, and smoking

status, a significant relationship between this genotype (*AA vs AG+GG*) with CAT score<10 for COPD risk was observed (AOR=2.70, p=**0.03**, 95% CI=1.10-6.66) (**Table 5.11**).

In the second group, individuals with a CAT score ≥10, even though both codominant and recessive models showed threefold risks for COPD, no significant association was observed in the genotypes for COPD susceptibility (**Table 5.11**)

Table 5.11: Association of *IL4RAQ576R* polymorphism and CAT score for COPD risk

CAT<10						
IL4RAQ576R	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	13 (6.43)	1.00 (reference)		1.00 (Reference)	-
AG	68 (33.49)	19 (9.40)	2.83 (1.32-6.08)	**0.007**	2.76 (1.12-6.80)	**0.02**
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	13(6.43)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	71 (34.97)	19 (9.40)	2.71 (1.26-5.82)	**0.01**	2.70 (1.10-6.66)	**0.03**
CAT≥10						
IL4RAQ576R	**Controls**	**Cases**				
Codominant	**n(%) N=203**	**n(%) N=202**	**OR[1](95%CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	132(65.02)	112(55.44)	1.00(Reference)	-	1.00 (Reference)	-
GG	3(1.47)	8 (3.96)	3.14 (0.81-12.13)	0.09	3.74 (0.45-30.56)	0.21
Recessive	**n(%) N=203**	**n(%) N=202**	**OR[1](95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA+AG	200 (98.52)	162 (80.19)	1.0 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	8 (3.96)	3.29 (0.85-12.61)	0.8	4.06 (0.53-30.69)	0.17

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 *IL4RAQ576R* polymorphism and mMRC for COPD susceptibility

The relationship between IL4RAQ576R polymorphism and mMRC was assessed in our research. Two groups were categorized based on the cut point of mMRC<2 and mMRC ≥ two as recommended by GOLD 2011 guidelines. In the first group of mMRC<2, in the

codominant model, the presence of heterozygous (*AG*)genotype was in 9.40% (19) cases and 33.49% (68) of healthy individuals. A significant two-fold increase in COPD risk was observed with the heterozygous (*AG*) genotype in comparison to the wild genotype (*AA*) (OR=2.83, 95%CI=1.32-6.08, p=**0.007**). The relationship remained substantial for COPD susceptibility when adjusted with other cofactors such as age, gender, and smoking status (AOR=2.76, 95% CI=1.12-6.80 p=**0.02**). The dominant model (*AA vs AG+GG*), combined heterozygous and mutant genotype (*AG+GG*), was observed in 9.40% of COPD individuals and 34.97% of healthy individuals. The incorporated association of *AG+GG* genotype for COPD susceptibility was strongly correlated (OR=2.71, 95%CI=1.26-5.82, p=**0.01**) when adjusted with parameters like age, gender, and smoking status, significant relationship between this genotype (*AA vs AG+GG*) with mMRC<2 for COPD risk was observed (AOR=2.70, p=**0.03**, 95% CI=1.10-6.66) as shown in below **Table 5.12**.In the second group, individuals having mMRC≥2, although the codominant and recessive models showed threefold risks for COPD, no correlation was observed in the genotypes for COPD risk (**Table 5.12**).

Table 5.12: Association of *IL4RAQ576R* polymorphism and mMRC for COPD risk

mMRC<2						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	13 (6.43)	1.00 (Reference)	-	1.00 (Reference)	-
AG	68 (33.49)	19 (9.40)	2.83 (1.32-6.08)	**0.007**	2.76 (1.12-6.80)	**0.02**
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	13 (6.43)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	71 (34.97)	19 (9.40)	2.71 (1.26-5.82)	**0.01**	2.70 (1.10-6.66)	0.03
mMRC≥2						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n(%) N=202**	**OR[1](95%CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	132(65.02)	112 (55.44)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3(1.47)	8 (3.96)	3.14 (0.81-12.13)	0.09	3.74 (0.45-30.56)	0.21
Recessive	**n(%) N=203**	**n(%) N=202**	**OR[1](95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA+AG	200(98.52)	162(80.19)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	8 (3.96)	3.29 (0.85-12.61)	0.08	4.06 (0.53-30.69)	0.17

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 *IL4RAQ576R* polymorphism and COPD severity

We have also assessed the relationship between COPD severity and IL4RAQ576R polymorphism. In the codominant model, the mutant (*GG*) genotype was observed in 0.99% of cases and 1.47% of controls. The association of mutant genotype *GG* compared to wild genotype *AA* for the susceptibility of COPD was insignificant (OR=2.66, 95%CI=0.42-16.61, p=0.29). Even when adjusted with other covariates like age, gender, and smoking status,

although there was a twelve-fold increase in the risk, the relationship of mutant genotype for COPD susceptibility was not substantial when compared to wild genotype (*AA*) (AOR=12.85, 95%CI=0.08-2022.81, p=0.32,). In the recessive model (*AA+AG vs GG),* the *GG* mutant

genotype also showed increased risk, but no significant correlation of this genotype was observed for COPD susceptibility compared to a combination of wild-type and heterozygous genotypes *AA+AG* (**Table 5.13**).

Table 5.13: Association of *IL4RAQ576R* polymorphism and COPD severity

COPD SEVERITY (YES)						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	33 (16.33)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	2 (0.99)	2.66 (0.42-16.61)	0.29	12.85 (0.08-2022.81)	0.32
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	45 (22.27)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	2 (0.99)	2.96 (0.48-18.25)	0.24	9.48 (0.17-509.07)	0.26

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 Correlation of COPD duration and *IL4RAQ576R* polymorphism

We investigated the association between the polymorphism of IL4RAQ576R and COPD duration (5-10 years). In the codominant model, the mutant (*GG)* genotype was found in 1.48% (3) COPD patients and 1.47% (3) healthy subjects. When compared to the wild-type genotype (AA), the mutant (*GG*) genotype showed a strong correlation with COPD duration of 5-10 years (OR=8.80, 95%CI=1.62-47.55, p=**0.01**). However, the risk decreased after adjusting it with parameters like age, gender, smoking status, and the association of mutant genotype (*GG*) compared to wild genotype (AA) for COPD duration of 5-10 years is insignificant. In the recessive model (*AA+AG vs GG),* the mutant *GG* genotype showed a seven-fold increase in risk among COPD individuals who had this disease for 5-10 years, and the relationship was significant (OR=7.40, 95%CI=1.42-38.57, p=**0.01**,) when compared to the combined wild and heterozygous genotypes (*AA+AG*). However, in the recessive model (*AA+AG vs GG),* the relationship ofthe *GG* genotype was not significant with COPD

duration after adjusting it with other cofactors like age, gender, and smoking status when compared to *AA+ AG* genotype (AOR=5.26, 95%CI= 0.33-82.64, p=0.23) as shown in **Table 5.14**

Table 5.14: Correlation of COPD duration and *IL4RAQ576R* polymorphism

DURATION OF COPD (5-10 YEARS)						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	15 (7.42)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	3 (1.48)	8.80 (1.62-47.55)	**0.01**	4.09 (0.13-128.45)	0.42
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	27 (13.36)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	3 (1.48)	7.40 (1.42-38.57)	**0.01**	5.26 (0.33-82.64)	0.23

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 Association of *IL4RAQ576R* polymorphism and COPD risk based on gender

Our study evaluated the relationship between IL4RAQ576R polymorphism and COPD risk based on gender. In the male category, the occurrence of mutant (*GG*) genotype was observed in 3.96% of COPD individuals and 0.49% of controls, whereas heterozygous (*AG*) genotype was found in 31.68% of cases and 17.73% in healthy subjects when compared to wild type genotype (*AA*) in the codominant model (OR= 5.73, 95% CI= 0.70-46.75, p=0.10). Although the mutant (*GG*) genotype showed some risk of COPD susceptibility, the association was insignificant. Also, when adjusted with covariates such as age, gender, and smoking status, the risk increased but again, the relationship of *GG* genotype for COPD risk in males was not substantial when compared to wild genotype (*AA*) (AOR= 6.40, 95% CI=0.38-105.25, p=0.19). In the dominant model (*AA vs AG+GG),* the combined heterozygous and mutant genotype (*AG+GG)* showed no significant correlation for COPD susceptibility in comparison to the wild genotype (*AA*) and although in the recessive model (*AA+AG vs GG),* the mutant

genotype showed five-fold risk but the relationship of *GG* genotype when compared to combined *AA+AG* genotypes for COPD risk was not significant. In the female category, no significant association was found in any of the models (codominant, dominant, and recessive) for COPD risk (**Table 5.15**).

Table 5.15: Association of *IL4RAQ576R* polymorphism and COPD risk based on gender

Males						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	81 (39.90)	113 (55.94)	1.00 (Reference)	-	1.00 (Reference)	-
AG	36 (17.73)	64 (31.68)	1.27 (0.77-2.09)	0.34	1.25 (0.61-2.58)	0.53
GG	1(0.49)	8 (3.96)	5.73 (0.70-46.75)	0.10	6.40 (0.38-105.25)	0.19
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	81 (39.90)	113 (55.94)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	37 (18.22)	72 (35.64)	1.39 (0.85-2.27)	0.18	1.38 (0.68-2.81)	0.36
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	117 (57.63)	177 (87.62)	1.00 (Reference)	-	1.00 (Reference)	-
GG	1 (0.49)	8 (3.96)	5.28 (0.65-42.83)	0.11	5.95 (0.43-80.67)	0.17
Females						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	51 (25.12)	12 (5.94)	1.00 (Reference)	-	1.00 (Reference)	-
AG	32 (15.76)	5 (2.47)	0.66 (0.21-2.06)	0.47	0.64 (0.17-2.32)	0.49
GG	2 (0.98)	0 (0)	-	-	-	-
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	51 (25.12)	12 (5.94)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	34 (16.74)	5 (2.47)	0.62 (0.20-1.93)	0.41	0.60 (0.16-2.19)	0.44
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	83 (40.88)	17 (8.41)	1.00 (Reference)	-	1.00 (Reference)	-
GG	2 (0.98)	0 (0)	-	-	-	-

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 Relationship of *IL4RAQ576R* polymorphism and GOLD severity for COPD risk

The association between polymorphism of IL4RAQ576R and GOLD 1234 was studied. Subjects were categorized into four groups based on spirometry: Group 1 included mild (FEV1≥ 80% predicted) patients, Group 2 included moderate (50% ≤FEV1 <80% predicted) cases of airway limitation, and Group 3 had severe (30% ≤FEV1 <50% indicated) and Group 4 included very strict (FEV1<30% predicted) cases of airflow obstruction.

In GOLD 1 and GOLD 2 categories, no significant relationship was found in any models towards susceptibility of COPD among all the patients and controls. In GOLD 3, in the recessive model (*AA+AG vs GG*), a strong correlation was observed between the mutant genotype (*GG*) in comparison to the combined *AA+AG* genotype for COPD risk (OR=4.32, 95%CI=1.01-18.55, p=**0.04**) However the association was not significant after adjusting it with various cofactors like age, gender, smoking status (AOR= 5.48, 95%CI=0.63-47.13, p= 0.12). In the codominant model, the mutant (*GG*) genotype showed no correlation with the wild-type genotype (*AA*) for COPD susceptibility. In the GOLD 4 category, in the codominant model (AA vs GG), the mutant genotype GG showed no significant association compared to wild genotype *AA.* In the recessive model (*AA+AG vs GG)*, the frequency of mutant genotype *GG* was observed in 0.49% of COPD individuals and 1.47% of healthy individuals. A protective effect was observed with mutant genotype *GG* when compared to the combined *AA+AG* genotype against COPD risk (OR=0.04, 95% CI= 0.00-0.34, p=**0.002**) and even after the adjustment with other parameters like age, sex, and smoking status, the relationship remained significant (AOR=0.02, 95% CI=0.00-0.26, p=**0.002**) (**Table 5.16**)

Table 5.16: Relationship of *IL4RAQ576R* polymorphism and GOLD 1234 for COPD risk

GOLD 1						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	5 (2.47)	1.00 (Reference)	-	1.00 (Reference)	-
AG	68 (33.49)	2 (0.99)	0.77 (0.14-4.10)	0.76	0.41 (0.05-2.97)	0.38
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	5 (2.47)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	71(34.97)	2 (0.99)	0.74 (0.14-3.93)	0.72	0.41 (0.05-2.94)	0.37
GOLD 2						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132(65.02)	40 (19.80)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	2 (0.99)	2.20 (0.35-13.63)	0.39	2.78 (0.22-34.69)	0.42
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	70 (34.65)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	2 (0.99)	1.90 (0.31-11.63)	0.48	2.2 (0.18-28.58)	0.52
GOLD 3						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	53 (26.23)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	5 (2.47)	4.15 (0.95-17.98)	0.057	5.31 (0.53-52.60)	0.15
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	77 (38.11)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	5 (2.47)	4.32 (1.01-18.55)	**0.04**	5.48 (0.63-47.13)	0.12
GOLD 4						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	27 (13.36)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	1 (0.49)	1.62 (0.16-16.26)	0.67	2.97 (0.00-1563.56)	0.73
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	40 (19.80)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	1 (0.49)	0.04 (0.00-0.34)	**0.002**	0.02 (0.00-0.26)	**0.002**

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

5.6.8. Association of *IL4RAQ576R* polymorphism and GOLD "ABCD" towards COPD susceptibility

According to GOLD 2011 guidelines, we classified our COPD cases into four groups: A, B, C, and D. We evaluated the association of *IL4RAQ576R* polymorphism and GOLD "ABCD" symptom-based assessment.

In category GOLD A, in the codominant model (*AA vs AG*),7.92% (16) COPD cases and 33.49% (68) healthy subjects had heterozygous (*AG*) genotype. A significant correlation was observed between *AG* genotype comparison to wild type genotype (*AA*) for COPD risk (OR=2.38, 95% CI=1.08-5.2, **p=0.03**); however, the association was not significant when adjusted with covariates like age, sex, smoking status (AOR=2.40, 95%CI= 0.96-6.00, p=0.06). In the dominant model *AA vs AG+GG,* the relationship of combined heterozygous and mutant (*AG+GG)* genotype in comparison to *AA* wild genotype for COPD susceptibility was significant (OR=2.38, 95% CI=1.08-5.2, **p=0.03**), nevertheless the correlation was insignificant after adjusting it with parameters like age, sex and smoking status as shown in **Table 5.17**.

No significant association was found in the GOLD B category, even though the risk was three-fold in both the codominant and recessive models (**Table 5.17**).

In the GOLD D category, no substantial relationship was observed. However, the risk associated with the mutant genotype (*GG*) in comparison to the wild genotype (*AA*) was eight-fold even after adjusting for cofactors like age, sex, and smoking status in the codominant model (AOR=8.73, p=0.36, 95% CI= 0.07-996.02). Also, the correlation was not significant in the recessive model even when the risk was ten-fold after adjusting it with various parameters such as age, gender, and smoking status (AOR=10.34, 95% CI=0.08-1236.63, p=0.33) as depicted in **Table 5.17**.

Table 5.17: Association of *IL4RAQ576R* polymorphism and GOLD ABCD for COPD risk

GOLD A						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	13 (6.43)	1.00 (Reference)	-	1.00 (Reference)	-
AG	68 (33.49)	16 (7.92)	2.38 (1.08-5.2)	**0.03**	2.40 (0.96-6.00)	0.059
Dominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	13 (6.43)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	71 (34.97)	16 (7.92)	2.28 (1.04-5.02)	**0.03**	2.35 (0.94-5.87)	0.06
GOLD B						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	98 (48.51)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	7 (3.46)	3.14 (0.79-12.46)	0.10	4.37 (0.51-36.83)	0.17
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	141 (69.80)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	7 (3.46)	3.30 (0.84-13.01)	0.08	4.53 (0.59-34.75)	0.14
GOLD D						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	132 (65.02)	14 (6.93)	1.0 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	1 (0.49)	3.14 (0.30-32.27)	0.33	8.73 (0.07-996.02)	0.36
Recessive	**n (%) N=203**	**n (%) N=202**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	200 (98.52)	21 (10.39)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3 (1.47)	1 (0.49)	3.17 (0.31-31.90)	0.32	10.34 (0.08-1236.63)	0.33

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

5.6.9 Association of *GSTM1/GSTT1* and *IL4RAQ576R* polymorphism for COPD risk

We evaluated the relationship between*GST(GSTM1/GSTT1*) polymorphism and *IL4RAQ576R* polymorphism for COPD susceptibility. In the case of the codominant model,

the prevalence of mutant type genotype (*GG*) was 4% in cases and 1% in controls who had *GSTM1(+).* No significant association was observed even though there was a three-fold risk (OR=3.7,p=0.10, 95%CI=0.76-17.99). After adjusting with covariates such as age, gender and smoking status, the association still remained non-significant when compared to wild genotype *AA* (AOR=2.99, p=0.35,95%CI=0.30-28.93). For the *GSTM1(-)* category, there was not any substantial correlation between *GSTM1(-)* and *IL4* polymorphism in all three models (Codominant, Dominant and Recessive) for COPD susceptibility. For *GSTT1(+),* no relationship was found between wild genotype *AA* and mutant genotype *GG* for COPD risk in codominant model(OR=1.9, p=0.36, 95%CI= 0.45-8.45; AOR=2.9, p=0.37, 95%CI=0.26-33.03). Also, there was no significant association observed between the null genotype of *GSTT1* and IL4 polymorphism for COPD susceptibility in the codominant model. Furthermore, in the dominant model (*AA vs AG+GG*), the combinatorial genotype (*AG+GG*) was found in 13% of cases and 4.5% of healthy subjects, but there was no significant relationship found between the polymorphism of IL4 and *GSTT1(-)* for COPD risk however after adjusting it with parameters like age, sex and smoking status, we found strong significant correlation of *GSTT1(-)* null genotype and the combinatorial genotype (*AG+GG*) for COPD susceptibility when compared to controls (AOR=3.49, **p=0.04**,95%CI=1.04-11.71) (**Table 5.18**).

Table 5.18: Association of *GSTM1/GSTT1* and *IL4RAQ576R* polymorphism for COPD risk

GSTM1(+) VS IL-4 polymorphism						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	75(37.5)	81(40.5)	1.00 (Reference)	-	1.00 (Reference)	-
GG	2(1)	8(4)	3.7(0.76-17.99)	0.10	2.99(0.30-28.93)	0.35
GSTM1(-) VS IL-4 polymorphism						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	51(25.5)	42(21)	1.00 (Reference)	-	1.00 (Reference)	-
AG	21(10.5)	31(15.5)	1.79(0.90-3.56)	0.09	2.12(0.68-6.55)	0.19
Dominant	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	51(25.5)	42(21)	1.00 (Reference)	-	1.00 (Reference)	-
AG+GG	22(11)	31(15.5)	1.7(0.86-3.38)	0.12	2.06(0.67-6.36)	0.20
Recessive	**n (%) N=200**	**n (%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA+AG	72(36)	73(36.5)	1.0 (Reference)	-	1.00 (Reference)	-
GG	1(0.5)	0(0)	4.2(0.87-20.19)	0.07	3.37(0.41-27.67)	0.25
GSTT1(+) VS IL-4 polymorphism						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n (%) N=200**	**n(%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	94(47)	80(40)	1.00 (Reference)	-	1.00 (Reference)	-
GG	3(1.5)	5(2.5)	1.9(0.45-8.45)	0.36	2.98(0.26-33.03)	0.37
GSTT1(-) VS IL-4 polymorphism						
Polymorphism **IL4RAQ576R**	**Controls**	**Cases**				
Codominant	**n(%) N=200**	**n(%) N=200**	**OR[1] (95% CI**	**P**	**AOR[2](95% CI)**	**P**
AA	32(16)	43(21.5)	1.00 (Reference)	-	1.00 (Reference)	-
AG	9(4.5)	23(11.5)	1.90(0.77-4.66)	0.15	3.36(0.99-11.37)	0.051
Dominant	**n(%) N=200**	**n(%) N=200**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	32(16)	43(21.5)	1.00 (Reference)	-	1.00(Reference)	-
AG+GG	9(4.5)	26(13)	2.14(0.88-5.21)	0.09	3.49(1.04-11.71)	**0.04**

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

DISCUSSION

Chapter 6

Discussion

Our lungs are exposed to pollutants, irritants, noxious particles, allergens, and pathogens every day, all of which can cause inflammatory responses and produce endogenous oxidants, leading to chronic inflammation, tissue injury, and remodelling. Surprisingly, the irritants listed above have been linked to COPD development and susceptibility. COPD is a multifaceted disease that results from the complex interplay of genetic and environmental factors. Inhaled xenobiotics are absorbed rapidly and detoxified by phase I and II enzymes, including glutathione-conjugating enzymes (GSTs) (van de Wetering *et al*., 2021). When GST gene variants are missing, related enzyme activity is lost, accumulating foreign particles, leading to severe problems like COPD or other diseases.

This experimental study employed the case-control research method, with 200 clinically evaluated COPD patients and 200 average healthy persons serving as control subjects. Our goal was to examine the role of GST polymorphism and clinical parameters in COPD risk. On average, COPD patients were 58±11.96 years old, while controls were 43.46 ±15.84. The study was conducted by Gaspar *et al.*, who published a mean age of 64.3 years for cases and 31.9 years for controls (Gaspar *et al.*, 2004). Dimov *et al.* conducted a study and found that the average age for patients was 67 years and 57 years for possession (Dimov *et al*., 2008)

The mean body mass index (BMI) for cases was 21.7 and 25.3 for controls. Cholera et al. reported a mean BMI of 19.7 in cases and 21.5 in healthy subjects (Arja *et al*., 2013). Begum *et al.* conducted another study that revealed a mean BMI of 19.7 among cases and a mean BMI of 28.4 among controls (Begum *et al*., 2011). The BMI of patients is lower than controls due to higher metabolism and reduced absorption. Nevertheless, the average metabolic rate in

our research remains within the normal BMI range, although still lower than in controls, which is possible because most of the cases in our investigation were ex-smokers.

The majority of the recruited cases for our study were ex-smokers; the mean pack-years in our report were 44.83 ± 39.58. Lakhdar *et al.*, and Begum *et al.*, discovered a mean pack-year of 51.8±29.4 and 88.2±19.8 (Lakhdar *et al*.,2010; Begum *et al*.,2011). However, Faramawy *et al.* and Mehrotra *et al*. reported that the average pack-years of COPD cases were 35.3±7.0 and 20.8±4.7respectively (Faramawy *et al*.,2009; Mehrotra *et al*.,2010). This demonstrates that fewer pack-years of smoking are related to COPD development.

Our study comprised of 200 COPD individuals out of which 7 (3.5%) were GOLD stage 1 (mild), 71 (35.5%) were GOLD stage 2 (moderate), 79 (39.5%) were GOLD stage 3 (severe), and 43 (21.5%) were GOLD stage 4 (very severe). Begum et al. reported similar findings, with mild COPD being reported in 73 (29.2%), moderate COPD in 48 (19.2%), severe COPD in 121 (48.4%), and very severe COPD in 8 (3.2%) (Begum *et al*., 2011). It suggests that COPD patients in India's tertiary hospitals are in the late stages of the disease, with severe symptoms. However, Ekberg-Aronsson et al. identified that mild COPD influenced 1779 people, moderate COPD influenced 1326 people, severe COPD influenced 197 people, and very severe COPD influenced 54 people in their investigation (Ekberg-Aronsson *et al*., 2005). It may be possible because patients in developed countries have better education, consciousness, and health infrastructure, resulting in earlier symptom detection and treatment.

In our report, 98% (196) of the patients complained of dyspnea, 19.5% (39) of dry cough, and 15.5% (31) of cough with expectoration. In a 2014 study, Zhang et al. observed that 16% (60) of patients had a dry cough, while 15.2 % (57) had a cough with expectoration (Zhang *et al*., 2014). In their investigation, there was no comparison of dyspnea. According to our findings,

the most common symptom was breathlessness, followed by a dry cough and a cough with expectoration.

The null genotypes for *GSTT1* and *GSTM1* are more common in some ethnic groups than others. Different metabolizing enzyme activities and types of dominant functional enzymes against oxidative stress may be linked to gene polymorphisms in different races (Cheng *et al*., 2004). COPD prevalence differences between ethnic groups are hard to differentiate from environmental factors (Yim *et al*., 2000). According to one study, 60% of Asians, 40% of Africans, and 20% of Caucasians have *GSTT1* enzyme deficiency (Strange *et al*., 1999). According to our findings, 34.5% of COPD patients (69 /200) have the *GSTT1(-)* genotype, compared to 20.5 % (41/200) of healthy people. We found a strong link between *GSTT1(-)* and COPD risk, and our findings are accurate to those of Nevzorova *et al*., Kukkonen *et al*., Mehrotra *et al*., and Thakur *et al*., (Nevzorova *et al*.,2012; Kukkonen *et al*., 2011; Mehrotra *et al*., 2010 and Thakur *et al*.,2011). According to the research above, the *GSTT1(-)* null genotype is a critical factor in COPD development. *GSTT1* and COPD were found to have no link in a few studies (Yim *et al*.,2000; Gaspar *et al*.,2004; Hemimi *et al*.,2008; Dimov *et al*.,2008; Zuntar *et al*.,2014). Although the *GSTM1(-)* genotype was found in 36.5% of cases and 36.5% of controls, no clear link between the *GSTM1(-)* null allele and COPD susceptibility was found. Our research showed that the *GSTM1(-)* null genotype is not a genetic marker for COPD. However, it is contradictory to the few studies done by various authors who claimed that the *GSTM1(-)* null genotype emerged to be a decisive risk factor for COPD (Dimov *et al*., 2008; Faramawy *et al*.,2009; Dey *et al*., 2014; Shukla *et al*., 2013 and Ahmad *et al*., 2016). The combined influence of *GSTM1(-)* null and *GSTT1(-)* null genotypes resulted in a two-fold increase in COPD risk, but the relationship was insignificant, similar to the findings of Mehrotra et al. (38). The *GSTM1(+)* and *GSTT1(-)* null genotypes were found to have a two-fold risk of COPD severity when combined.

The link between GST polymorphism and the mMRC scale and CAT score is reported for the first time in our study. The *GSTT1* null genotype strongly correlated with mMRC for COPD susceptibility. It means that people who do not have the *GSTT1* gene are nearly four times more likely than healthy people to develop escalating breathlessness. The *GSTT1(-)* null gene variant is significantly associated with COPD risk as measured by the CAT score. It implies that COPD patients with the *GSTT1(-)* gene have a higher risk of worsening symptoms than those without it.

In our investigation, there were 298 (74.5%) males and 102 (25.5%) females. We wanted to see if there was a link between the GST polymorphism and COPD risk based on gender. The *GSTT1(-)* null allele was linked to COPD susceptibility in males and females. Females with the *GSTT1(-)* null genotype were more vulnerable than males with the same gene deletion. According to a systematic review of 11 studies, female smokers had a faster annual decline in FEV1 than male smokers amidst smoking fewer cigarettes (Gan *et al*., 2006). COPD is 50 % more likely to strike women than in men. COPD, unlike emphysema, is linked to indoor air pollution and contributes to minor airway disorders, which demonstrates why women are more likely to develop this risk factor (Assad *et al.,* 2015). Females are more prone to COPD than males because their airways are smaller for the same lung volume, resulting in a higher concentration of tobacco smoke per unit area of small airway surface (Merkus *et al*., 1996). Tam and colleagues used a mouse model exposed to chronic cigarette smoke to investigate the possibility that female sex hormones contribute to females' increased COPD susceptibility (Tam *et al*., 2016).

Our report looked at the link between GST polymorphism and GOLD severity and discovered a strong correlation between *GSTT1(-)* null genotype and GOLD severity based on FEV1 values. There was no relation between the *GSTM1(-)* null genotype and GOLD severity for COPD risk. COPD patients with the *GSTT1(-)* null genotype are more susceptible

to airway obstruction than those with the *GSTM1(-)* null genotype. Nevertheless, a study published by Khan et al. postulated that *GSTM1(-)* null genotype prevalence occurred significantly in individuals with severe COPD (Ahmad *et al*., 2016).

An association between the GST polymorphism and the GOLD "ABCD" symptom-based assessment was explored in this study. We discovered a strong link between the *GSTT1(-)* null allele and the GOLD B category for COPD susceptibility. It signifies that COPD patients have a high symptom severity but a low risk of exacerbation. This correlation has never been studied before, and more data is needed to understand it better.

Our findings revealed a significant relation between *GSTT1(-)* and COPD duration, implying that patients with COPD have lost *GSTT1* gene activity and are more vulnerable to disease severity for extended periods. To learn more about the above relationship, a study should be conducted.

More focus on treatment and prevention is needed with the increased incidence of COPD and high mortality rates worldwide. Currently, no medication is the most effective in treating COPD-related pulmonary damage. Genetic markers can help detect diseases early on, treating them more effectively. In order to find a suitable genetic quality for COPD in North Indian patients, we conducted research. The *GSTT1(-)* null genotype, according to our findings, can be used as a genetic marker for COPD risk. *GSTT1* deletion has also been linked to COPD severity, breathlessness exacerbation, symptoms worsening, and other clinical parameters.A more extensive population study will be necessary to assess the relationship between the *GSTT1* homozygous null gene and other xenobiotic enzymes as risk variables for COPD progression and pathogenesis.

Complex host–environment interactions that occur over time can lead to COPD. Due to genetic predisposition and weakened immunity, smoking and other pollutants, pathogens, and

allergens harm the lungs, causing airway inflammation and damage in a susceptible host (Scambler *et al*., 2018) which results in permanent damage, a fixed airflow obstruction, and the subsequent typical COPD symptoms. The inflammatory response involves innate and adaptive immunity in COPD (Brightling *et al.,* 2019). By enlisting, activating, and encouraging the survival of numerous inflammatory cells in the respiratory tract, cytokines play a significant role in orchestrating the chronic inflammation associated with COPD (Barnes *et al.,* 2008). IL4 is a pleiotropic cytokine that has a significant aspect in regulating inflammation. Overexpression of IL4 is thought to cause significant mucus metaplasia, an increase in mucin expression and goblet cells (Chung *et al.,* 2001), an increase in the release of chemokines like CCL11 and the expression of adhesion molecules like VCAM-1 on lung fibroblasts, inducing swelling of the airway, and inhibition of eosinophil and Th2 lymphocyte apoptosis by Bcl-2 protein expression (Vandenbroeck *et al*.,2003). Hence it becomes mandatory to determine the role of IL-4 polymorphism in COPD risk as this disease is majorly characterised by inflammation.

We employed case-control research to evaluate the significance of *IL-4* polymorphism and clinical parameters of COPD. In this study, no significant association was found between *IL4RAQ576R* polymorphism and COPD risk even though codominant (*AA vs GG*) and recessive models (*AA+AG vs GG*) exhibit some risk toward COPD, but the relationship was not at all substantial. This SNP has not been studied widely concerning COPD risk; hence we could not correlate our findings with other studies. However, there are heterogeneous findings related to *IL-4* gene variants and COPD susceptibility. Hegab *et al*. found no significant difference in the genotype frequencies of *IL-4* and *IL-13* polymorphisms between the COPD cases and healthy subjects (Hegab *et al*., 2004). Also, a study by Tang *et al.* demonstrated no association of *IL-4 -589C/T* polymorphism with COPD risk (Tang *et al*.,2016). However, research done by Chi *et al*. showed a statistically significant difference in

*IL-4-33, IL-4-590*and *IL-41098* alleles between COPD patients and ordinary Hebei people, so it can be inferred that *IL-4-33, IL-4-590* and *IL-4-1098* alleles may be candidates for COPD patients (Chi *et al*.,2018) Also, *IL-4* polymorphism is mainly studied for the pathogenesis of bronchial asthma (Li *et al.,* 2016; Hegab *et al*., 2004; Beghe *et al*.,2003; Rosa-Rosa *et al.,*1999).

The link between *IL4RAQ576R* polymorphism and the mMRC scale and CAT score is studied for the first time in our study. In the case of CAT<, 10, the codominant (*AA vs GG*) and dominant (*AA vs AG+GG*) models for *IL4RAQ576R* polymorphism showed a significant correlation for COPD susceptibility. It means that COPD people with the heterozygous *AG* genotype or combined heterozygous or mutant *(AG+GG)* genotype are two times more likely than healthy people to develop worsening symptoms. In the case of CAT≥10, the risk of having breathlessness was higher; however, the association was not substantial for COPD susceptibility. In the case of mMRC<2, the codominant (*AA vs GG*) and dominant (*AA vs AG+GG*) models for *IL4RAQ576R* polymorphism showed a significant association for COPD risk. It implies that COPD people with the heterozygous (AG) genotype or combined heterozygous or mutant (*AG+GG*) genotype are two times more prone to escalating breathlessness than healthy controls. In the case of mMRC≥2, the risk of having breathlessness was higher, but the association of *IL4RAQ576R* polymorphism was not at all substantial for COPD susceptibility.

We also assessed the association between COPD severity and *IL4RAQ576R* polymorphism and found no significant association even though the risk was higher in both the codominant and recessive models. Our findings revealed a significant relationship between *IL4RAQ576R* and COPD duration, implying that COPD patients with mutant genotype (GG) are more vulnerable to disease severity for extended periods. To learn more about this association, a study should be conducted. We also investigated if there was a link between the *IL-4* gene

variant and COPD risk based on gender. No significant correlation was found even though the COPD risk associated with males was higher than females in all the three models (Codominant, Dominant, Recessive).

Our study evaluated the link between *IL4RAQ576R* polymorphism and GOLD severity and discovered a significant correlation between the recessive model of GOLD stage 3 based on FEV1 values and *IL4RAQ576R* polymorphism, implying that COPD patients who have severe airway obstruction are more likely to have increased inflammation and therefore more prone to COPD susceptibility. However, a significant protective effect was observed between the *IL4RAQ576R* polymorphism and GOLD stage 4 patients in the recessive model (*AA+AG vs GG*). Further studies need to be done to confirm this finding. An association between the *IL4RAQ576R* polymorphism and the GOLD "ABCD" symptom-based assessment was explored in this study. We discovered a strong link between the GOLD A in codominant (*AA vs AG*) and dominant (*AA vs AG +GG*) model and *IL-4* gene variant. It signifies that COPD patients have a low symptom severity and moderate exacerbation. However, other GOLD stages such as B and D exhibit higher risks, but the association was not at all substantial. The association of GOLD "ABCD" with *IL4RAQ576R* polymorphism for COPD risk has never been assessed before, and more studies are needed to better understand it.

We evaluated the relationship between *GST(GSTM1/GSTT1*) polymorphism and *IL4RAQ576R* polymorphism for COPD susceptibility. We observed that cases having GSTT1(-) genotype were linked to the IL4RAQ576R polymorphism after adjusting it with confounding factors such as age, sex and smoking status in the dominant model (*AA vs AG+GG*). It means that people with COPD who lack the GSTT1 gene may accumulate xenobiotic or endobiotic toxins, which then trigger the inflammatory process, an immune reaction to harmful noxious particles which can be abnormal over the period if toxins are persisting in the lungs or any other organ. However, we could not find a significant

relationship between *GST M1(+/-)* and *GSTT1(+)* genotype with *IL4RAQ576R* polymorphism, even though the risks were higher for COPD susceptibility.

COPD is a heterogeneous disorder controlled by various genes and environmental factors. Numerous studies have been done to find the genetic biomarker for this complex disease, except that only the α- 1 antitrypsin gene is widely associated with COPD (Menga *et al*.,2020). The other candidate genes differ significantly across regions and races. Human *IL-4* is thought to play a crucial role in the pathogenesis of asthma disease because it can initially encourage B lymphocytes to produce IgE. *IL-4*, which may play a role in the pathogenesis of COPD, can either directly or indirectly activate the stimulation of inflammatory cell aggregation-induced airway hyperresponsiveness, according to relevant research and experiments. Even though *IL-4* may play a role in the pathogenesis of COPD and asthma, there are still few domestic studies in this area.

Additionally, the results can vary depending on the sample size, variable factors, geographical effects, and other factors (Barnes *et al.*,2013; Vogelmeier *et al*., 2013; Celli *et al*., 2015). At this time, SNPs of *IL-4-33C/T*, *-589C/T*, and other loci have been the subject of in-depth research on genetic polymorphisms. According to Vanfleteren *et al*. (2013), the presence of COPD may be associated with the *IL-4* haplotype (-589C, -33C). According to Khnlein *et al*. (2014), the heterozygous genotype of the *IL-4* promoter region-*589C/T* may be connected to the prevalence of COPD. There are comparatively more studies on the variations in *IL-4* levels in COPD patients, but there are still few studies on *IL-4* gene polymorphisms, particularly those with a large sample size of a particular regional variable. Foreign research on this gene has also produced a range of findings, which may be related to regional variations (Thomsen *et al.*, 2013; Barrecheguren *et al.*, 2015). It may be noted that a more extensive population study will validate and assess the role of *IL4RAQ576R* polymorphism in COPD susceptibility, pathogenesis and progression.

CONCLUSION

Chapter 7

Conclusion

In conclusion, our study demonstrated that *GSTT1* polymorphism play a pivotal role in contributing to increased susceptibility of Chronic Obstructive Pulmonary Disease (COPD) in North Indian population. Only the combination of *GSTM1(+)/GSTT1(-)* genotype showed significant correlation towards COPD risk. However, *GSTM1* polymorphism does not give the substantial contribution to COPD development and pathogenesis.

On the other hand, the inflammatory cytokine*IL-4R(α)* polymorphism (*IL4RAQ576R*) also did not show significant relationship with COPD risk. However, it exhibited substantial link with some of the clinical parameters pertaining to COPD.

To establish a predictive genetic biomarker for each individual, subgroups, or different ethnic groups in support of the prevention scope, validation of these findings with larger population studies and more rigorous study design are required.

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APPENDIX

1. **0.5 M EDTA**: Dissolved 9.306g of disodium salt of EDTA in 20 ml of deionised water, and then adjusted the pH to 8.0 by 1M sodium hydroxide. Sterilized the solution by autoclaving.
2. **10% SDS**: Dissolved 1g of SDS in 10 ml of deionised water.
3. **100mM Tris - Cl** (pH 8.0): Dissolved 0.32g of Tris - Cl in 10 ml of deionised water, then adjusted the pH to 8.0 by 1M sodium hydroxide. Sterilized the solution by autoclaving.
4. **10mg/ml Proteinase K**: Dissolved 10 mg Proteinase K in 1ml of double distilled water. Sterilized the solution by autoclaving.
5. **1mg/ml BSA**: Dissolved 100mg of BSA in 100 ml of deionised sterile water and kept at 4^0C overnight.
6. **5M Sodium chloride** (NaCl): Dissolved 5.85g of sodium chloride in 20 ml of deionised water. Sterilised the solution by autoclaving.
7. **5X TBE buffer**: Dissolved 54 g of Tris base and 27.5 g of boric acid in 980 ml of double distilled water and then added 20 ml of 0.5M EDTA. Sterilized the solution by autoclaving.
8. **Ethidium Bromide**(10mg/ml): Dissolved 1g of ethidium bromide in 100 ml of water. Mixed the solution properly.
9. Magnesium chloride ($MgCl_2$)(100mM): Dissolved 0.41 g of $MgCl_2$ in 20 ml of deionised water and sterlised by autoclaving.
10. **Sucrose**(1M): Dissolved 3.41 g of sucrose in 10ml of deionised water and sterilized by autoclaving.
11. **TE buffer** (pH 8.0):Added 1ml of 100mM Tris – Cl (pH 8.0) and 200 µl of 0.5M EDTA solution to 8.8 ml of deionised water. Sterilise the solution by autoclaving.
12. **Triton X-100**(10%): Took 100µl of Triton X-100 and mixed with 900 of deionised water and mixed properly.

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1.	PBY 205	Advanced Plant Biotechnology	A
2.	PBY 209	Animal Cell Culture and Transgenic Technology	A
3.	PBY 208	Bioinformatics & System Biology	A
4.	PBY 202	Biopharmaceutical & Pharmaceutical Technology	A
5.	PBY 201	Downstream Processing	A
6.	PBY 305	Drug Design & Development	A
7.	PBY 104	Applied Immunology & Vaccine Technology	A-
8.	PBY 102	Bioprocess Engineering	A-
9.	PHU 301	Entrepreneurship & IPR	A-
10.	PBY 207	Food Processing Technology	B
11.	PBY 101	Molecular Biology & rDNA Technology	A-
12.	PMA 102	Research Methodology	B-

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