Evaluation of Oxidant-Antioxidant Imbalance, Level of Nitrite, and Level of Catalase as Biomarker in Plasma Samples of Chronic Obstructive Pulmonary Disease (COPD) Patients

A Thesis

submitted in partial fulfillment of the requirements for the award of Degree of

Master of Science

in

Biotechnology

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

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JULY 2023

DECLARATION

I hereby declare that work done in the seminar report entitled, "**Evaluation of Oxidant-Antioxidant Imbalance, Level of Nitrite, and Level of Catalase as Biomarker in Plasma Samples of Chronic Obstructive Pulmonary Disease (COPD) Patients**" submitted towards partial fulfilment of requirement for award of **Master of Science degree** in Biotechnology in **Department of Biotechnology** at **Thapar Institute of Engineering and Technology, Patiala**, is an authentic record of work carried out by me under the supervision and guidance of Dr. Siddharth Sharma, Associate Professor and under the co-supervision and co-guidance of Dr. Sanjai Saxena, Professor at the Department of Biotechnology in Thapar Institute of Engineering and Technology, Patiala.

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

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DATE: 28/07/2023

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

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This is to certify that dissertation entitled, "**Evaluation of Oxidant-Antioxidant Imbalance, Level of Nitrite, and Level of Catalase as Biomarker in Plasma Samples of Chronic Obstructive Pulmonary Disease (COPD) Patients**" submitted by **Ms. Pratibha Kalra** in partial fulfilment of the requirements for the award of M.Sc. in Biotechnology at Thapar University, Patiala is an authentic work carried out by her under my supervision and guidance.

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

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ACKNOWLEDGEMENT

This report is completed with prayers of many and love of my family and friends. However, there are a few people that I would like to specially acknowledge and extend my heartfelt gratitude who have made the completion of this report possible. With the biggest contribution to this report, I would like to thank Associate Professor **Dr Siddharth Sharma** and Professor **Dr Sanjai Saxena**, they had given me their full support in guiding me with stimulating suggestions and encouragement to go ahead in all the time of the report.

I am grateful to all the faculty members of the Department of Biotechnology for their help and encouragement. I also place on record, my sense of gratitude to one and all who, directly or indirectly, have lent their helping hand in this venture. Indeed the words to my command are not adequate either in form or in spirit to convey my in depth gratitude to **Ms. Ramandeep Kaur**, **Ms. Shreya Gupta, Ms. Heena Kansal**, **Ms. Anmol Bhatia**, and **Ms. Nidhi Mahajan** for their valuable guidance, expedient advice and affection during the course of investigation and preparation of this manuscript.

I would also like to thank **my parents** and **my brother** for their years of unyielding love and encouragement.

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LIST OF ABBREVATIONS USED

S.NO.	ABBREVATIONS	FULL FORM
1	COPD	Chronic Obstructive Pulmonary Disease
2	GOLD	Global Initiative for Chronic Obstructive Lung Disease
3	FEV_1	Forced Expiratory Volume in 1 second
4	FVC	Forced Vital Capacity
5	GOLD1	According to the GOLD system, mild airflow limitation
6	GOLD2	According to the GOLD system, moderate airflow limitation
7	GOLD3	According to the GOLD system, severe airflow limitation
8	GOLD4	According to the GOLD system, very severe airflow limitation
9	ROS	Reactive Oxygen Species
10	MMPs	Matrix metalloproteinases
11	NAC	N-acetylcysteine
12	SNP	Single-nucleotide polymorphism
13	ELN	Elastin
14	TB	Tuberculosis
15	WHO	World Health Organisation

16	BODE	Body mass index, degree of obstruction, dyspnea, and exercise capacity
17	TAC	Total antioxidant capacity
18	TPTZ	Tripyridyltriazine
19	OS	Oxidative stress
20	DPPH	2,2-diphenyl-1-picrylhydrazyl
21	FRAP	Ferric Reducing Antioxidant Power
22	NO	Nitric oxide
23	NOS	Nitric oxide synthase
24	MDA	Malondialdehyde
25	SOD	Superoxide Dismutase
26	CAT	Chronic obstructive pulmonary disease (COPD) assessment test (CAT)
27	BSA	Bovine serum albumin

Abstract

This dissertation aims to investigate potential biomarkers for Chronic Obstructive Pulmonary Disease (COPD) sensitivity, focusing on oxidative stress and antioxidant capacity. Three specific biomarkers were evaluated: Total Antioxidant Capacity (TAC) measured through 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays, Nitrite levels using the Griess assay, and Catalase activity through Catalase In-Gel Activity Assay. The study enrolled 160 participants, including COPD patients and healthy controls. The DPPH assay revealed no statistically significant difference in TAC between COPD patients (mean 10.12 ± 0.04 μg/ml) and healthy controls (mean 11.03 ± 0.02 μg/ml). Additionally, TAC did not significantly differ among COPD patients based on age, gender, smoking status, and COPD severity. The FRAP assay, however, indicated a significantly higher TAC in COPD patients (mean 57.02 ± 0.27 μg/ml) compared to healthy controls (mean 23.43 ± 0.22 μg/ml).

Furthermore, the Griess assay demonstrated a lower concentration of nitrites in the plasma of COPD patients compared to healthy controls. While no significant differences in nitrite levels were observed among different subgroups of COPD patients based on age, smoking status, COPD severity, and symptoms (CAT scores), the findings suggest an alteration in the nitric oxide pathway in COPD. The Catalase In-Gel Activity Assay revealed lower catalase activity in COPD patients compared to healthy controls. This finding implicates a potential link between reduced catalase activity and increased oxidative stress in COPD patients, contributing to disease pathogenesis. The study's implications suggest the importance of exploring oxidative stress and antioxidant capacity in COPD management. Identifying reliable biomarkers for COPD sensitivity can aid in disease diagnosis and personalized treatment approaches. However, the study has certain limitations, including the cross-sectional design, small sample size, and potential confounding factors like diet and lifestyle choices. In conclusion, this dissertation provides valuable insights into potential biomarkers for COPD sensitivity related to oxidative stress and antioxidant capacity. The results shed light on the complexity of antioxidant status in COPD, warranting further research with larger cohorts and comprehensive assessments. Exploring the impact of specific antioxidant interventions and conducting longitudinal studies can enhance understanding and contribute to novel therapeutic strategies for COPD management.

CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Chronic Obstructive Pulmonary Disease (COPD)

Global Initiative for Chronic Obstructive Lung Disease (GOLD) has described Chronic Obstructive Pulmonary Disease (COPD) as "a common preventable and treatable disease that is characterized by **persistent airflow limitation** (Fig. 1.1). It is usually **progressive** and associated with an enhanced chronic inflammatory response in the airways and the lungs to noxious particles or gases. Exacerbations and co-morbidities contribute to the overall severity in individual patients" (Vestbo *et al*., 2013).

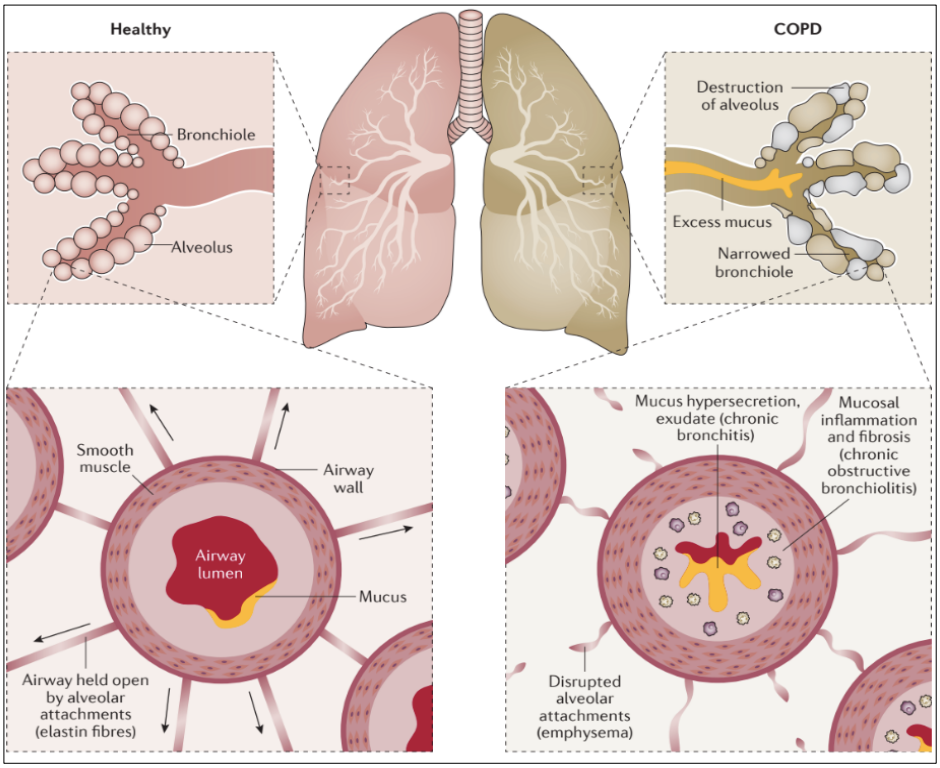


Figure 1.1 Airway obstruction in COPD (Barnes *et al*., 2015)

COPD is a significant public health issue globally. It is one of the top five leading causes of death globally and ranked fifth worldwide concerning disease burden (Lozano *et al*., 2013). Smoking is the leading cause of COPD, especially in high-income nations. However, several additional risk factors have been discovered in low-income nations (Yawn *et al*., 2009). COPD is most prevalent in people above the age of forty. Growing research suggests a connection between chronic inflammatory diseases and aging. COPD is a chronic inflammatory disorder of the lungs, it progresses very slowly, and most patients are elderly (Ito & Barnes, 2009).

In COPD, airway obstruction (Fig. 1.1) progresses slowly. With age, lung function typically declines. The normal deterioration in lung function is accelerated in COPD patients.

Progressive airflow limitation eventually leads to shortness of breath on exertion, also known as dyspnea. Dyspnea eventually leads to exercise restriction, which lowers the quality of life (Lozano *et al*., 2013). There is mounting evidence that faulty endogenous anti-aging mechanisms cause the lung parenchyma to age more quickly (Ito & Barnes, 2009) and emphysema is brought on by the activation of pathways that cause telomere shortening and cellular senescence (Mercado *et al*., 2015). The chronic inflammation linked to COPD is typically resistant to corticosteroids (Barnes, 2013). The imbalance between proteinases and anti-proteinases (Barnes *et al*., 2003) and the imbalance between oxidant and antioxidant (Wada & Takizawa, 2013) are also major contributing factors to the pathophysiology of COPD, in addition to inflammatory responses (Barnes *et al*., 2003).

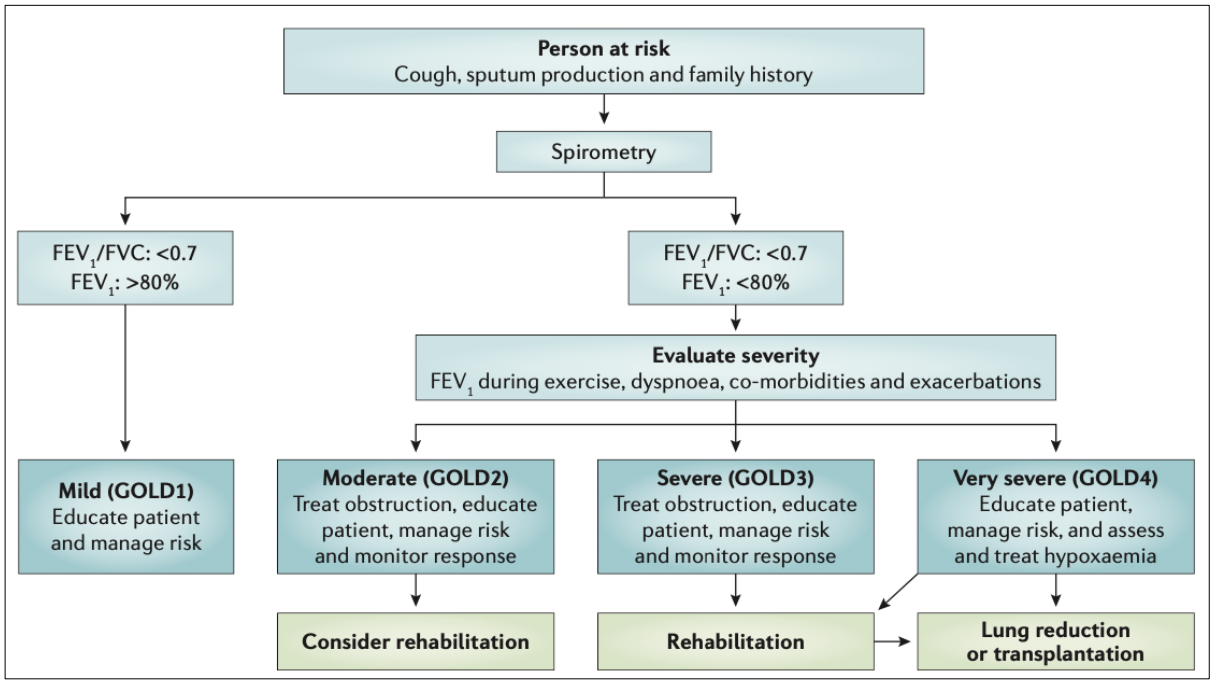


Figure 1.2 | Algorithm for the diagnosis, staging and management programme for COPD (Barnes *et al*., 2015)

Due to the lung's structural changes, the airflow obstruction in COPD is typically irreversible. Airflow restriction is interpreted to prevail when the FEV_1 to FVC ratio is less than 0.7 where FVC is forced vital capacity, and FEV_1 is forced expiratory volume in one second. The GOLD system has categorized airflow limitation into mild, moderate, severe and very severe (Fig. 1.2). In patients with $FEV_1/FVC < 0.7$:

- GOLD1 - Mild airflow limitation: has an FEV_1 of >80% of predicted

- GOLD2 - Moderate airflow limitation: has an FEV_1 of 79-50% of predicted
- GOLD3 - Severe airflow limitation: has an FEV_1 of 49-30% of predicted
- GOLD4 - Very severe airflow limitation: has an FEV_1 of <30% of predicted

Mostly in healthy people, FEV_1 is greater than 70% (>0.7) (Lange *et al*., 2015).

1.2 Oxidant and antioxidant imbalance (oxidative stress) in COPD

Under normal physiological conditions, a balance exists between oxidants and antioxidants, and here oxidants are reactive oxygen species produced during normal cellular metabolism. Antioxidants protect cells from damage caused by reactive oxygen species (ROS) or free radicals. Free radicals interact with antioxidants and get stabilized, which mitigates some of the potential harm that free radicals could have done. The imbalance (Fig. 1.3) between the generation of reactive oxygen species and the antioxidant capacity leads to oxidative stress. The pathogenesis of several human diseases, including COPD, has been related to this imbalance. Furthermore, it has been indicated that the pathophysiology of COPD is greatly influenced by the imbalance of oxidants and antioxidants (Elmasry *et al*., 2015). However, the precise mechanism of the etiopathogenesis of COPD is not yet well defined.

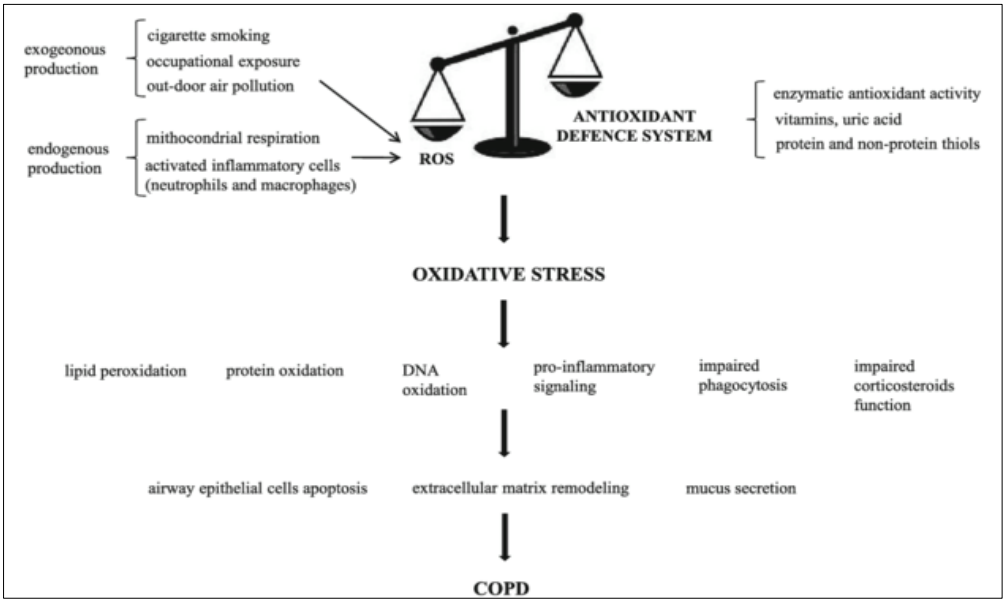


Figure 1.3 | Mechanism showing the central role of oxidative stress in the pathophysiology of COPD (Zinellu *et al*., 2016)

In COPD, exposure to cigarette smoke increases oxidative stress exogenously, while activation of inflammatory cells increases oxidative stress endogenously (Caramori *et al*., 2011). Increased oxidative stress is one of the main driving factors in the pathophysiology of COPD and is also responsible for many of its features (Kirkham & Barnes, 2013). Additionally, oxidative stress causes DNA damage that is not repaired by DNA repair machinery in COPD patients. It has been reported that this failure to repair double-stranded DNA break might increase the risk of developing lung cancer (Caramori *et al*., 2011).

CHAPTER 2

REVIEW OF LITERATURE

2. Review of Literature

2.1 Chronic Obstructive Pulmonary Disease (COPD)

2.1.1 Chronic Inflammation and COPD

The hallmark of COPD (chronic obstructive pulmonary disease) is chronic inflammation. The body's normal response to injury or infection is inflammation, characterized by an influx of immune cells, the release of inflammatory mediators, and changes to the tissue. However, with COPD, this inflammation persists and speeds up the course of the illness. Chronic inflammation primarily impacts lung tissue and airways in patients with COPD. Inhaling irritants, especially tobacco smoke, causes an immunological reaction that attracts immune cells to the lungs, including neutrophils, macrophages, and T-lymphocytes. The pro-inflammatory mediators these immune cells release include cytokines, chemokines, and enzymes like matrix metalloproteinases (MMPs).

Chronic inflammation in COPD results in several damaging effects

Airway inflammation, mucus hypersecretion, tissue destruction, and oxidative stress in COPD. Chronic inflammation in COPD is sustained by ongoing exposure to irritants, such as smoking, air pollution, and occupational exposures. The inflammatory response becomes dysregulated, with an imbalance between pro-inflammatory and anti-inflammatory factors. This dysregulated inflammation perpetuates tissue damage and contributes to disease progression. Managing chronic inflammation is an essential aspect of COPD treatment. Strategies include smoking cessation. The most effective way to reduce inflammation in COPD is to quit smoking. Avoiding exposure to tobacco smoke is crucial for preventing further lung damage and inflammation.

There is increasing evidence that in COPD there is an association between airflow obstruction and abnormal inflammatory response of the lungs. This chronic inflammatory response of the lungs results from chronic inhalation exposure to smoke, dust particles and other air pollutants. This chronic inflammation further results in the loss of elasticity of the lungs (Panzner, 2002). Emerging evidences have shown that occurrence of COPD and tobacco smoking addiction are closely corelated (Tam & Sin, 2012). It has been reported that in the course of COPD, ROS, which plays an essential role in the oxidant-antioxidant imbalance, is derived from inflammation-inducing cells such as neutrophils and macrophages (MacNee & Rahman, 2000).

Chronic inflammation is a hallmark of COPD, contributing to airway obstruction, tissue destruction, and oxidative stress. Managing inflammation through various interventions is essential for slowing disease progression, improving symptoms, and enhancing the overall well-being of individuals with COPD.

2.1.2 ROS and COPD

ROS (Reactive Oxygen Species) and COPD (Chronic Obstructive Pulmonary Disease) are relevant topics in respiratory health. ROS are chemically reactive molecules that contain oxygen. They are generated as natural by-products of various cellular processes, including aerobic metabolism. Examples of ROS include superoxide anion ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$). ROS play a crucial role in cellular signalling and defence against pathogens in normal physiological conditions. However, excessive ROS production or impaired antioxidant defence mechanisms can lead to oxidative stress, causing damage to cells, tissues, and organs.

COPD is a progressive lung disease characterized by persistent airflow limitation and airway inflammation. The primary risk factor for COPD is tobacco smoking, although exposure to air pollutants and genetic factors can also contribute to its development. The two main conditions that fall under COPD are chronic bronchitis and emphysema. Chronic bronchitis involves long-term inflammation of the bronchial tubes, leading to increased mucus production and coughing. Emphysema, on the other hand, is characterized by the destruction of the lung tissue, specifically the alveoli, responsible for gas exchange. Oxidative stress, resulting from an imbalance between ROS production and the body's antioxidant defence mechanisms, is believed to play a significant role in the pathogenesis and progression of COPD. In individuals with COPD, ROS production is increased in the lungs due to several factors, including cigarette smoke exposure, inflammation, and cellular damage. These ROS can cause direct damage to lung tissues, initiating a cascade of inflammatory responses.

The excessive production of ROS in COPD can activate various signalling pathways, leading to the release of pro-inflammatory cytokines and chemokines, recruitment of immune cells, and tissue remodelling. This chronic inflammation and oxidative stress contribute to the progressive decline in lung function observed in COPD patients. Furthermore, ROS can also inactivate natural antioxidant defences in the lungs, such as glutathione and superoxide dismutase, further exacerbating oxidative stress. Various treatment strategies are employed to

manage COPD and mitigate the effects of oxidative stress. These include smoking cessation, pharmacological interventions (bronchodilators, corticosteroids, etc.), pulmonary rehabilitation, and supplemental oxygen therapy when necessary. Antioxidant therapies, such as N-acetylcysteine (NAC), have also been investigated to counteract the effects of ROS in COPD, although their clinical efficacy is still debatable.

One of the significant causes of cell and tissue damage associated with COPD is reactive oxygen species (ROS). It has been reported that cigarette smoke directly and the release of increased ROS indirectly result in the airway leucocytes in the blood or air spaces responsible for generating increased oxidative stress (Rahman *et al*., 2000). It has been reported that antioxidants are depleted by ROS. It has been observed that the concentrations of ascorbate and vitamin E are depleted among smokers. It has been reported that in comparison to non-smokers, smokers are 15-20% less ascorbate concentration in their serum (Calverley *et al*., 2023). In summary, ROS and oxidative stress play a crucial role in the development and progression of COPD. Managing oxidative stress and reducing the production of ROS are essential aspects of COPD treatment and can help alleviate the symptoms and slow disease progression.

2.2 Causes and risk factors

2.2.1 Smoking

Smoke from cigarettes is the primary source of oxidative free radicals (Burrows et al., 1977). Because it causes an oxidant excess in the lower airways, cigarette smoke is the primary etiological factor in the pathophysiology of COPD. Per puff, cigarette smoke comprises 4700 compounds, including peroxynitrite, superoxide radicals, nitrogen oxides, and 1016-1017 oxidant molecules (Rajendrasozhan *et al*., 2009).

As not all smokers get COPD, cigarette smoke has a significant negative impact, but other risk factors also play a significant role. Under typical circumstances, various extracellular and intracellular antioxidants properly guard the blood and lungs against the harmful effects of oxidants (Heffner & Repine, 1991). In smokers and COPD patients, there is evidence of an imbalance between oxidants and antioxidants in the blood and lungs (Tavilani, 2012). Lipid peroxidation is one of the effects of increased ROS formation (Fig. 1.3).

2.2.2 Passive smoking

It has been reported that both tobacco smoking and environmental exposure to smoking are leading causes of COPD (Rajendrasozhan *et al*., 2009). It passively gets exposed to different kinds of smoke at various places like homes, workplaces or public areas. It can also be referred to by various other terms – second-hand smoking, environmental tobacco smoking and side-stream smoking.

This may make sense in light of the relative health consequences and exposures to various amounts of cigarette smoke, outdoor air pollution, and inside air pollution, this may make sense (Burnett *et al*., 2014). Some experimental data suggests that measures lessened indoor pollution exposure and slowed lung function loss (Zhiu *et al*., 2014). Airway damage and remodelling are caused by inflammation and oxidative stress, which can occur in response to inhaled irritants.

2.2.3 Genetic factors

Several studies have shown that COPD tends to cluster in families and is heritable in many cases (Zhou *et al*., 2013). α1-antitrypsin deficiency and cutis laxa increase COPD risk (Silverman & Sandhaus, 2009). α1-antitrypsin deficiency is caused due to homozygosity of an SNP (single-nucleotide polymorphism) of an allele, namely, PI*Z allele. PI*Z is an allele of the α1-antitrypsin gene called SERPINA1 (Molloy *et al*., 2014). There is emerging evidence that heterozygosity of PI*Z in individuals and smoking increases the risk of COPD (Sorheim *et al*., 2010). Cutis laxa is caused by gene mutation, including elastin (ELN). Elastin is a significant component of the lung extracellular matrix (Rodriguez-Revenga *et al*., 2004). Not all smokers develop COPD, and other factors, such as genetics and epigenetics, cancontribute to the development and progression of the disease.

2.2.4 Socioeconomic status

According to the data provided by the Office of Population Censuses and Surveys, a strong association between mortality and COPD has been suggested. A notably higher death rate for COPD has been recorded for countries with low per capita gross national income. This also explains the higher number of death cases in East and South Asia. This trend was also observed in the United Kingdom, where a strong association was noted between COPD deaths and socioeconomic status. It was even highlighted that the impact of socioeconomic status was way more pronounced in COPD than in tuberculosis and lung cancer.

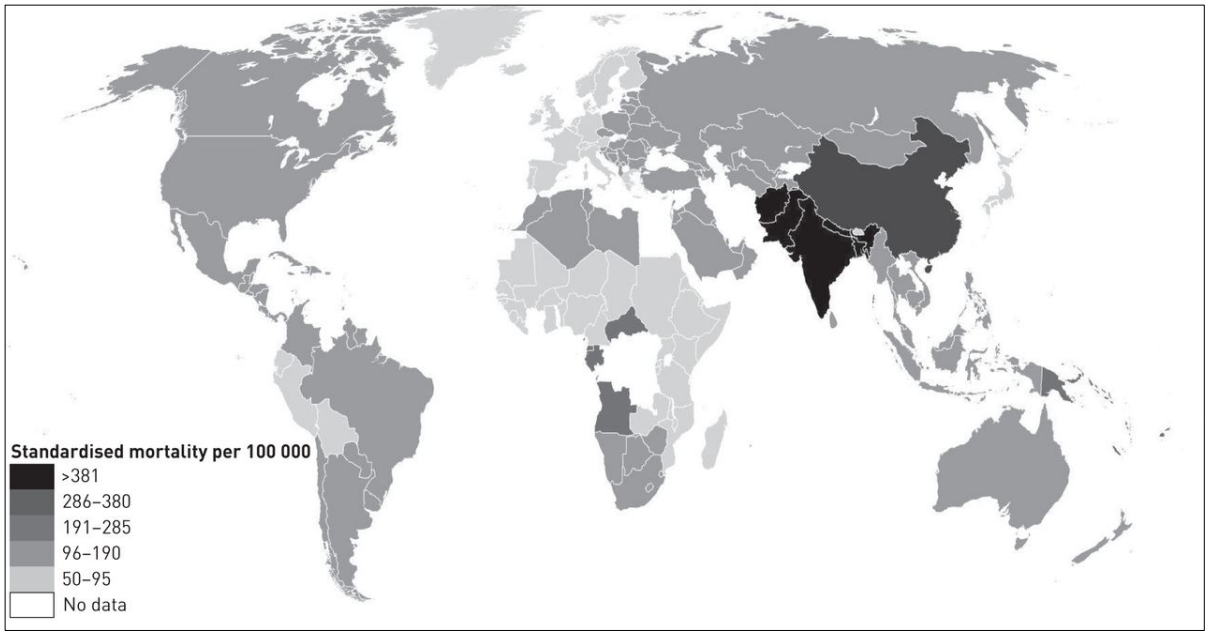


Figure 2.1 Age- and sex-adjusted mortality rate for COPD (Burney *et al*., 2015)

A strong association between low FVC and per capita gross national income has also been reported (Burney *et al*., 2014). The role of less medication uptake has also been highlighted as a reason for more deaths in poorer countries due to COPD. However, given the efficacy of the treatment, the association between the less uptake of medication and more COPD deaths in poorer countries was found to be less likely (Gnatiuc *et al*., 2015).

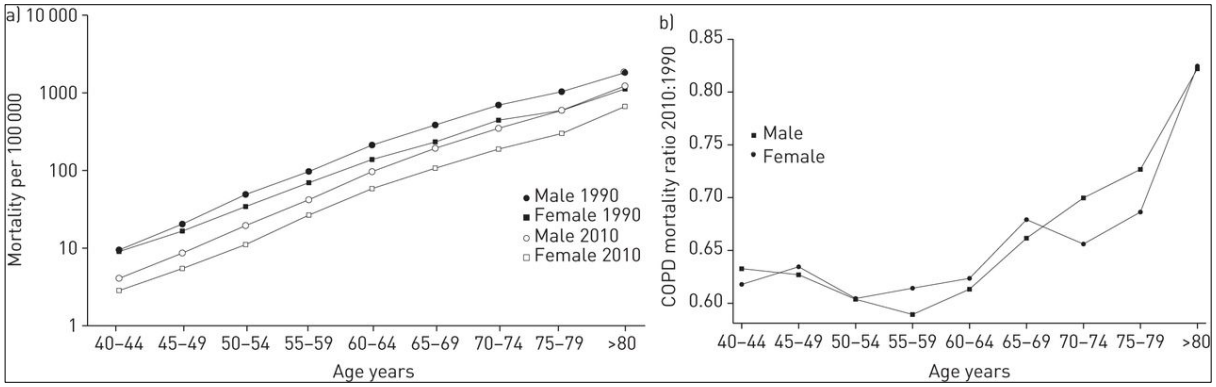
2.2.5 History of Tuberculosis

After smoking and exposure to environmental tobacco smoke, the next major risk factor for COPD is a history of tuberculosis infection (Menezes *et al*., 2007). TB-associated COPD or post tubercular airway disease affects a significant portion of TB patients (Snider *et al.,* 1971). Numerous other associations have also been reported:

- Furthermore, COPD alters the clinical course of TB and increases the risk of both morbidity and mortality from the condition (Leung *et al*., 2003)
- COPD is the second most common comorbidity among TB patients, behind diabetes (Wang *et al*., 2005)
- Pulmonary TB is most likely to affect COPD patients (Lee *et al*., 2013)
- A history of tuberculosis has a major impact on the long-term course of COPD, leading to earlier death and more exacerbations (Yakar *et al*., 2017)

2.2.6 Age

COPD is primarily a disease of elderly subjects (Ito & Barnes, 2009). A strong association has been seen between COPD death rates and increasing age. More COPD deaths have also been attributed to the ageing population (Burney *et al*., 2015). The disease involves accelerated ageing of the lungs and, unsurprisingly, becomes more common as people age, with a peak prevalence in those 65 years of age.



(a) (b)

Figure 2.2 Mortality rates and mortality ratio of COPD; a) Chronic obstructive pulmonary disease (COPD) mortality rates for 1990 and 2010, and b) COPD mortality ratios for 2010:1990 according to sex and Age (Burney *et al*., 2015)

2.2.7 Gender

Although historically, men were more likely than women to develop COPD as they were more likely to smoke, recent figures indicate that men and women have a similar risk of developing the disease. An estimated 20-30% of smokers develop COPD. There is minimal evidence to suggest that areas where biomass fuels are widely used experience significant rates of chronic airflow obstruction. Additionally, even in these areas, airflow obstruction is frequently more common in males than women, even though men would be expected to be exposed to indoor biomass at a higher rate.

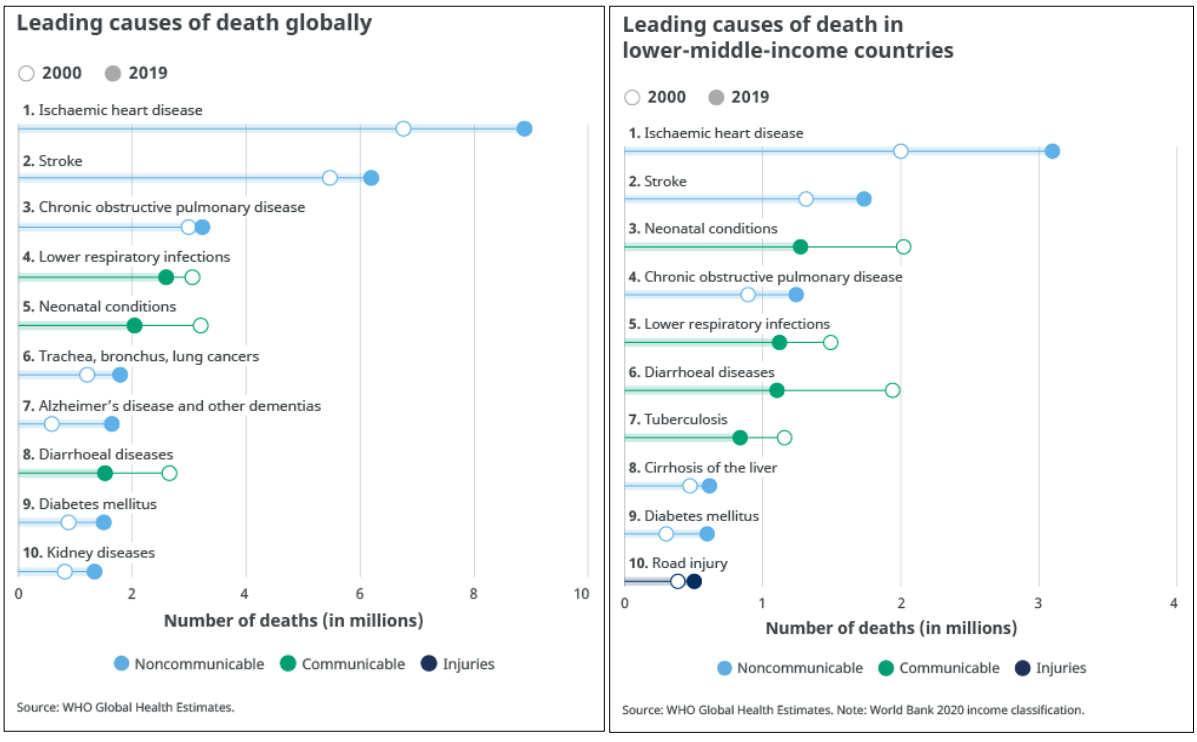
When 'European' groups are removed from the research, the relationship between socioeconomic position and COPD mortality strengthens, especially in women because smoking is a less significant confounder in their community. The link of COPD mortality with

poverty among the other nations after eliminating the European countries supports the idea that the association is less likely to be explained on ethnic grounds because European populations appear distinct. As the smoking habit has increased in younger individuals and the prevalence of COPD in women is now approaching or has surpassed (in some developed countries) that of men, COPD is now prevalent in younger individuals and particularly in women, who might develop the disease at an earlier age than men (Cote & Chapman, 2009).

2.3 Epidemiology

2.3.1 Scenario of COPD Worldwide

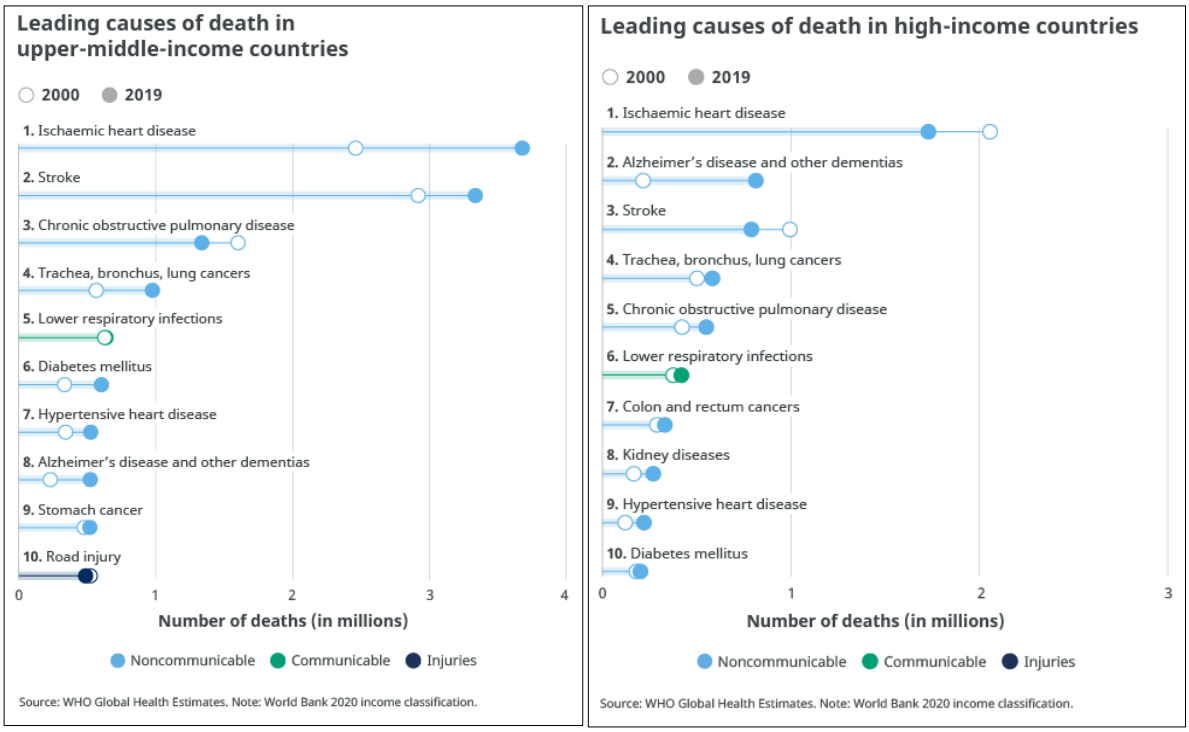
According to the data by WHO, COPD is the third leading cause of death globally and poses a significant public health issue. The most comprehensive data on the global distribution of COPD come from the mortality statistics compiled by the WHO and the Global Burden of Disease Programme. These data show that "COPD" (in which COPD was listed as the cause of death rather than diagnosed using the GOLD standards) was the third-most common cause of death in 2010.



(a) (b)

Figure 2.3 Leading causes of cancer; a) Leading causes of death globally, and b) Leading causes of death in lower & middle-income countries (WHO Global Health Estimates)

It has been reported that East and South Asia account for most COPD deaths. This could be because much proportion of the world's population resides in this region. Surprisingly, it has been observed that the death rates in East and South Asia showcase a high age-standardization. Two trends have been reported for COPD death rates, men compared to women have higher death rates, and with increased age, the death rates rise exponentially. In continuation to the latter trend, it has been reported that COPD, as the leading source of death, has been sourced in the ageing of the world's population over the past 20 years (Burney *et al.*, 2015).



(a) **(b)**

Figure 2.4 Leading causes of death; a) Leading causes of death in middle & upper-income countries, and b) Leading causes of death in high-income countries (WHO Global Health Estimates)

It has been reported that the regions highlighted for having the most tobacco consumption are not the same as those with the highest COPD death rates. To resolve the same, a hypothesis was put forth, which stated that high death cases in low and middle-income countries were attributed to heavy exposure to smoke from biomass burning (Salvi *et al.*, 2009). The most common causes of chronic airflow obstruction worldwide are smoking and exposure to tobacco smoke (Buist *et al.*, 2007), followed by a history of tuberculosis (Menezes *et al.*, 2007) and then dusty work environments in association with poverty and lack of education about the

disease risk (Hooper *et al*., 2012). Emerging pieces of evidences suggest that lowering indoor pollution exposure (Romieu *et al*., 2009) results in lower lung function rates (Zhou *et al*., 2014).

A European study suggested a strong correlation between the death rate and outdoor air pollution from coal burning. The United Kingdom introduced a Clean Air Act, after which the correlation faded over the years. However, it was suggested that the correlation might be valid in regions with high levels of air pollution from similar sources (Chinn *et al*., 1981).

2.3.2 Scenario of COPD in India

COPD has been ranked as the second leading cause of death in India (https://www.healthdata.org/india). This also explains why East and South Asia account for most COPD deaths (Fig. 2.5).

2.4 Clinical Features and Diagnosis

Some of the symptoms of COPD include:

- difficulty breathing
- shortness of breath
- reduced exercise capacity
- muscle weakness
- exacerbations
- airway narrowing
- emphysema
- chronic bronchitis
- cough
- expectoration of sputum

- lower respiratory tract infections occurring more frequently or lasting longer than expected (>2 weeks)

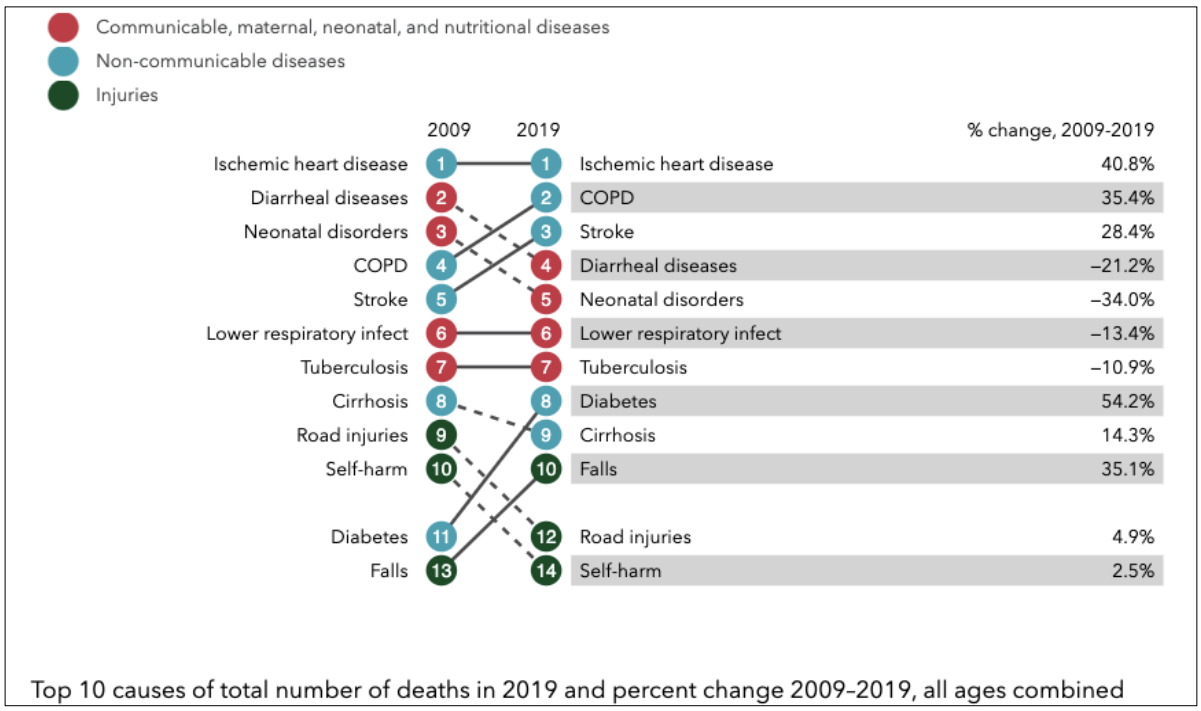


Figure 2.5 Top 10 causes of the total number of deaths in 2019 and percent change from 2009 to 2019, all ages combined (https://www.healthdata.org/india)

2.5 COPD Screening

2.5.1 Clinical presentation

The most significant sign of respiratory compromise, dyspnea, can be functionally graded to help stage the severity of the condition, raise the level of suspicion, and instruct the healthcare professional to perform a spirometry test (Vestbo et al., 2013). The Modified Medical Research Council dyspnoea scale is one such scale. It ranges from 0 to 4, with the lowest grade meaning no dyspnoea with activity and the highest grade implying dyspnoea with little exercise (Celli & MacNee, 2004). The presence of rhonchi (rattling sounds), coarse crackles and wheezes, increased respiratory rate with forced expiratory efforts, decreased breath sounds on chest auscultation (listening), and, in the most severe cases, cyanosis (blue skin discolouration, a sign of hypoxaemia) might be present in patients with more advanced disease and should be considered a critical complication requiring oxygen therapy. It is currently possible to identify hypoxaemia early because to the simplicity of pulse oximetry, a non-invasive way of monitoring oxygen saturation. For patients who have saturations below 88% when breathing

room air, oxygen should be provided. (Vestbo *et al*., 2013). Cor pulmonale is characterized by severe dyspnea, decreased exercise tolerance, leg oedema, and, in the most severe cases, generalized oedema (failure of the right side of the heart due to hypoxaemia and increased intrapulmonary vascular resistance). The majority of COPD patients combine characteristics of the traditional groupings of the "pink puffer" (Burgel *et al*., 2012) and the "blue bloater" phenotypes (Garcia-Aymerich *et al*., 2011). according to the results of a CT scan of the thorax and biomarkers. Compared to blue bloaters, having a higher body mass index, less emphysema, more metabolic co-morbidities, and cardiac compromise, pink puffers have less muscle mass, more emphysema, and fewer cardio-vascular and metabolic co-morbidities.

2.5.2 Confirming the Diagnosis

Documentation of expiratory airflow restriction during a forced expiration manoeuvre from total lung capacity to residual volume is required to diagnose COPD. This measurement is accomplished by utilizing a straightforward spirometer and recording the timed FVC as per established guidelines (Miller *et al*., 2005). As previously indicated, most healthy people exhale more than 70% of their vital capacity in the first second of the manoeuvre. Patients with COPD and restrictive pulmonary illnesses typically have poor FEV_1. As a result, a decline in the ratio of FEV_1/FVC to a value typically 0.7 is necessary for the diagnosing COPD. To distinguish between the significant reversibility that characterizes airflow restriction in individuals with asthma and the poorly reversible airflow limitation caused by COPD, the spirometry test should be performed following the administration of inhaled bronchodilators. Because elderly, otherwise healthy persons may have FEV_1/FVC levels of 0.7, the precise criteria of what constitutes an unequivocal diagnosis of COPD is still up for debate. The overdiagnosis of airway obstruction in the elderly is reduced when the FEV_1/FVC ratio is calculated using the lower limit of average rather than the fixed ratio to solve this issue (Swanney *et al*., 2008). As a result, there should be both a low FEV_1 and a lowered FEV_1/FVC compared to reference values found in population studies. (Hankinson *et al*., 1999). The current GOLD scale classifies the severity of airflow obstruction as a percentage of normal FEV_1.

The assessment of patients with COPD may be complemented by further research on lung function. When employing a sealed body box for body plethysmography, many patients, particularly in advanced illness stages, shall have higher lung volumes and air trapping. Exercise shall worsen hyperinflation, and the amount of air trapped correlates well with the severity of dyspnea. The ability of carbon monoxide to diffuse into the blood is a proxy for the

lung's ability to permit gas movement through the alveoli. Low readings for the diffusion capacity indicate emphysema or pulmonary vascular impairment and can aid in the early detection of the disease.

2.6 Staging

Numerous recommendations for COPD's thorough assessment have been produced in recognition of the condition's many facets. The body mass index, degree of obstruction, dyspnea, and exercise capacity (BODE) index and its variants, such as the BODEx (where the rate of exacerbations substitutes the exercise), is the most commonly appraised of these assessments (Celli et al., 2004). Other assessments include the age, dyspnoea and obstruction (ADO) index (Puhan et al., 2009), and the dyspnoea, obstruction, smoking and exacerbation (DOSE) index (Jones et al., 2009). More accurately than the straightforward FEV_1 measurement, all these markers predict mortality. Finally, GOLD has suggested a grading system that divides patients into A, B, C, or D groups based on obstruction, symptoms, and exacerbations. Preliminary research using mortality as the endpoint has revealed that the above grading system is not superior to the previous one, which just took into account FEV_1, in any way (Marin et al., 2013).

2.7 Treatment and Therapy

There is no reversal of the airway damage caused by COPD. Treatment seeks to reduce symptoms, slow the spread of the disease, and lower the chance of exacerbations, during brief episodes of symptom worsening typically brought on by viral or bacterial lung infections. Reducing exposure to airborne irritants, especially tobacco smoke, can improve the course of disease. In addition to pulmonary rehabilitation, which can enhance exercise capacity, lessen shortness of breath, and enhance general health status, bronchodilators are used to treat the symptoms of COPD (Barnes & Adlock, 2009).

Smoking cessation is the only treatment option to stop the further progression of COPD. However, exacerbation risk could be reduced using long-acting bronchodilators and influenza vaccination. To treat exacerbations, antibiotics and oral corticosteroids can be used. Bronchodilators can help reduce symptoms of COPD but cannot treat the underlying cause of COPD; hence bronchodilators cannot stop disease progression (Hansel & Barnes, 2009).

The following have been used to help manage chronic inflammation:

- Medications: Inhaled corticosteroids and bronchodilators are commonly prescribed to reduce airway inflammation, alleviate symptoms, and improve lung function. These medications help control inflammation and dilate the airways, making breathing easier.

- Pulmonary rehabilitation: This multidisciplinary program includes exercise training, breathing techniques, and education to improve physical conditioning and reduce symptoms. It can also help manage inflammation and improve overall quality of life.

- Oxygen therapy: In cases of severe COPD, supplemental oxygen may be prescribed to relieve hypoxemia (low blood oxygen levels) and reduce inflammation associated with tissue hypoxia.

- Avoidance of triggers: Minimizing exposure to environmental irritants, such as air pollutants and occupational hazards, can help reduce inflammation and prevent exacerbations (Barnes, 2013).

Research to clarify the cellular and molecular causes of COPD might lead to new medicines that target specific disease processes and halt or even reverse airway damage. Furthermore, the reasons why only a portion of chronic smokers develop the disease are largely unclear, and fuller knowledge of the contribution of other risk factors to COPD aetiology might lead to improved screening and prevention techniques. Finally, while being a prevalent disease, COPD is still not well understood; raising knowledge of this condition could lead to an earlier diagnosis and better patient outcomes (Kirkham & Barnes, 2013).

2.8 Potential Biomarkers for COPD

2.8.1 Total antioxidant capacity (TAC), oxidative stress and COPD

It has been reported that gender and smoking have discriminating effects on oxidant and antioxidant imbalance. It has been observed that serum levels of all biomarkers of oxidative stress are higher in males than females. There has been increasing evidences that females in comparison to males have lower level of antioxidant enzymes. Higher incidence of smoking and greater external exposure have been reported as the main factors behind this difference. Very few studies have even highlighted that sex hormones may have a role. It has been reported that smoking patients with COPD have lower levels of antioxidant enzymes and higher levels of oxidative stress markers than non-smokers (Burrows *et al*., 1977).

Non-smoking patients with COPD develop oxidative stress from sources other than cigarette smoking, such as respiratory infections, inflammation, dust and air pollution. It has been reported that the continuation of oxidative stress results from the reduction in naturally occurring antioxidants. The increased oxidative stress biomarkers in COPD patients have a role in pathogenesis. Cough signified increased oxidative stress and reduced antioxidants (Waseem *et al*., 2012). The FRAP assay is based on the reduction of a Fe^{3+} complex of tripyridyltriazine (Fe $(TPTZ)^{3+}$) to Fe $(TPTZ)^{2+}$ which is intensely in blue colour at low pH. Excess Fe^{3+} is utilized and $Fe(TPTZ)^{2+}$ is the rate-limiting factor. Thus the colour formation reflects the reducing ability of the sample (Hsieh & Rajashekaraiah, 2021).

The ferric reducing ability of plasma (FRAP) assay determines a given sample's total antioxidant capacity (TAC). This study attempts to analyze the possibilities of FRAP as an indicator of oxidative stress biomarkers in plasma samples of COPD patients. The ferric-reducing ability of plasma (FRAP) is an assay used to measure the antioxidant power. However, FRAP was developed to give a more biologically relevant overview than individual biomarkers of oxidative stress (OS). Antioxidants (endogenous and exogenous) together provide protection against reactive oxygen species (ROS) than individual compounds. Therefore overall antioxidant capacity, such as FRAP, gives a cumulative effect of all the antioxidants present than individual proportional to the concentration of the electron-donating antioxidants.

FRAP can be used as a single test for the estimation of the total antioxidant capacity of the blood. FRAP describes the prooxidant–antioxidant equilibrium better than other assays. FRAP does not measure thiol antioxidants and the reduction of ferric ions. However, FRAP has gained importance as it is simple, cost-effective, straightforward, fast, and highly reproducible compared with other tests of total antioxidant capacity. During blood storage, OS is induced, which causes irreversible damage that limits its shelf life. OS represents an imbalance between the ROS produced and the biological system's ability to counteract or detoxify the ROS or repair the resulting damage caused. Blood and its components possess an innate antioxidant system that helps in components.

FRAP is the only assay that measures the antioxidants directly compared with other assays that measure the inhibition of free radicals. FRAP is directly protecting itself against ROS. Since plasma holds all the blood's cellular components in the suspension, it provides an overview of the OS microenvironment over storage. Free radicals are highly unstable molecules that can

cause OS, triggering cellular damage. Antioxidants combat these free radicals, thereby providing a protective effect. An antioxidant is “any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate.”

2.8.2 Nitrite and COPD

Nitric oxide (NO) production is thought to increase during inflammatory immune processes involving the respiratory tract. However, this comes at a cost because too much NO can harm the respiratory system and thus contribute to the pathophysiology of inflammatory airway diseases like COPD and asthma (Kharitonov, 1994). Excessive production of NO has been reported in asthmatic airways (Vliet, 1999), although its presence was controversial in COPD airways. NO, a molecule and a potent free radical influences numerous facets of pulmonary function in healthy individuals and patients. It is produced from the amino acid L-arginine by the three different types of nitric oxide synthase (NOS). NO can be inhaled, converted to nitrite and nitrate through metabolism, or combined with super-oxide to generate peroxynitrite after being produced (Rutgers, 1999). Smoking control subjects, patients with stable or worsened illness, and patients who recovered from an exacerbation all had identical serum nitrate concentrations. In contrast to smoking control persons, patients with stable and worsened COPD had higher serum nitrite concentrations (Csoma *et al.,* 2019).

2.8.3 Catalase and COPD

In COPD patients, the antioxidant levels of catalase and SOD are much lower. Several investigations have indicated that COPD patients had much higher levels of MDA and lower levels of catalase and SOD than healthy controls (Tavilani et al., 2012). With higher GOLD stages came a considerable reduction in catalase and SOD levels (Waseem et al., 2012).

2.9 Gaps in the Literature

There is a need for more research to understand the underlying disease mechanisms and disease endotypes, as well as to identify useful biomarkers and develop new therapeutic and management approaches.

CHAPTER 3

AIM OF STUDY

3. Aim of study

This dissertation is aimed to evaluate the following:

3.1 Levels of Total Antioxidant Capacity (TAC) in patients with Chronic Obstructive Pulmonary Disease (COPD) and Healthy Controls as biomarker for COPD sensitivity using DPPH and FRAP Assay

3.2 Levels of Nitrite in patients with Chronic Obstructive Pulmonary Disease (COPD) as biomarker for COPD Sensitivity using Griess Assay

3.3 Levels of Catalase using Catalase In-Gel Activity Assay in Patients with Chronic Obstructive Pulmonary Disease (COPD) and Healthy Controls as a Biomarker for COPD Sensitivity

CHAPTER 4

MATERIALS AND METHODS

4. Materials and methods

4.1 Sample collection

This study accounts for 100 COPD patients and 60 healthy controls from the Department of Pulmonary Medicine, Government Medical College (GMC), Patiala, India. This study has been reviewed and approved by the Institute Ethics Committee of Government Medical College (GMCP), Patiala and Thapar Institute of Engineering and Technology. For the subjects diagnosed with COPD, an informed written consent was obtained from them or their representatives. Each patient completed a detailed questionnaire containing information on demographic and smoking characteristics such as tobacco habits like smoking beedi/cigarette etc. The subjects having a regular smoking habit were classified as smokers. The details such as CAT score, mMRC score, GOLD severity, GOLD group, smoking period, FEV value, symptoms and biomass exposure of patients were obtained from the hospital records. Gender and other parameters were also taken into consideration. Approximately 3-5 ml of venous blood was collected from each participant.

4.2 Sample preparation

4.2.1 Plasma isolation

From whole blood samples of COPD patients, plasma was isolated. The whole blood was centrifuged at 2000 g for 15 minutes at 4°C. The supernatant (plasma) obtained was isolated and stored (Ashworth *et al*., 2021).

4.3 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Fresh DPPH radical solution was prepared by dissolving 0.1 mM of DPPH in 100 ml of 70% (v/v) methanol. 10µl plasma samples were added to a methanolic solution of DPPH. Then it was centrifuged for 5 min at 14,000 g to remove precipitated protein and incubated for 25 minutes. The supernatant was added to a 96-well plate the absorbance was measured using a microplate reader at 517 nm. TAC was expressed as µg/ml equivalent to ascorbic acid (Lee *et al*., 2017).

Table 4.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

1.	490 µl DPPH methanolic solution was added to 10 µl plasma
2.	Incubated the solutions containing the sample for 25 minutes at 21°C
3.	Centrifuged at 14,000 g for 5 minutes
4.	Measured the absorbance at 517 nm

To obtain the standard curve, 0.1 mM methanolic solution of ascorbic acid was used as a standard antioxidant solution. 10 µl of different concentrations of methanolic solution of ascorbic acid from 0 µg/ml to 25 µg/ml were added to 490 µl of DPPH solution. 10 µl of 70% methanol was added to 490 µl of DPPH solution was used as blank. 500 µl DPPH solution was used as control.

Table 4.2 Different concentrations of antioxidant solution for standard curve

S. No.	Concentration of Ascorbic Acid (µg/ml)	Ascorbic acid solution (µl)	70% methanol (µl)	DPPH solution (µl)
1.	0	0	10	490
2.	5	1.25	8.75	490
3.	10	2.50	7.50	490
4.	15	3.75	6.25	490
5.	20	5.00	5.00	490
6.	25	6.25	3.75	490

For conducting the DPPH assay, freshly prepared DPPH solution was used every time. To obtain the DPPH solution following were added:

- 0.1 mM DPPH solution

0.1 mM DPPH solution was prepared by adding 3.94 mg 2,2-Diphenyl-1-picrylhydrazyl to 70% methanol (70 ml methanol + 30 ml distilled water).

4.4 Ferric Reducing Antioxidant Power (FRAP) Assay

Fresh FRAP working reagent was prepared by dissolving 25 ml of 300 mM of acetate buffer, 2.5 ml of 10 mM 2,4,6-tripirydyl-S-triazine and 2.5 ml of 20 mM $FeCl_3.6H_2O$. 10 µl plasma sample was added to 300 µl. Then it was incubated for 5 minutes at 37°C. Then it was added in a 96-well plate and the absorbance was measured using a microplate reader at 593 nm. TAC was expressed as µg/ml equivalent to ascorbic acid (Olszowy-Tomczyk *et al*., 2021).

Table 4.3 Ferric Reducing Antioxidant Power (FRAP) Assay	
1.	300 µl FRAP working reagent was added to 10 µl plasma
2.	Incubated the solutions containing the sample for 5 minutes at 37°C
3.	Measured the absorbance at 593 nm

To obtain the standard curve, 1 mM aqueous ascorbic acid solution was used as a standard antioxidant solution. 10 µl of different concentrations of an aqueous ascorbic acid solution from 0 µg/ml to 100 µg/ml were added to 300 µl of FRAP working reagent. 10 µl of distilled water added to 300 µl FRAP working reagent was used as blank.

Table 4.4 Different concentrations of antioxidant solution for standard curve				
S. No.	Concentration of Ascorbic Acid (µg/ml)	Ascorbic acid solution (µl)	Distilled water (µl)	FRAP working reagent (µl)
1.	0	0	10	300
2.	20	2	8	300
3.	40	4	6	300

4.	60	6	4	300
5.	80	8	2	300
6.	100	10	0	300

For the FRAP assay, a freshly prepared FRAP working reagent was used every time. To obtain FRAP working reagent following were added:

- 25 ml of 300 mM of acetate buffer,
- 2.5 ml of 10 mM 2,4,6-tripirydyl-S-triazine and
- 2.5 ml of 20 mM $FeCl_3.6H_2O$

Table 4.5 FRAP working reagent

Stock solution	**Working solution**
1 M Sodium Acetate	0.3 M Sodium Acetate
17.4 M Acetic Acid	0.3 M Acetic Acid
100 mM 2,4,6-tripirydyl-S-triazine	10 mM 2,4,6-tripirydyl-S-triazine
12 M HCl	40 mM HCl
100 mM $FeCl_3.6H_2O$	20 mM $FeCl_3.6H_2O$

300mM of acetate buffer was prepared by adding 0.3 M acetic acid dropwise to 50 ml 0.3 M sodium acetate until the desired pH was obtained i.e., 3.6. The working solution of sodium acetate with 0.3 M concentration was prepared from the stock solution of 1 M. To make the stock solution of 1 M, 1.6406 g sodium acetate was dissolved in 20 ml of distilled water.

Industry-standard acetic acid having a 17.4 M concentration was diluted, and the desired concentration of acetic acid was obtained, i.e., 0.3 M. To prepare 0.3 M acetic acid, 0.86 ml of 17.4 M acetic acid was added to a measuring cylinder, and volume was made up to 50 ml.

The working solution of 2,4,6-tripirydyl-S-triazine having 10mM concentrations was prepared from the stock solution of 100 mM. To make the stock solution of 100 mM, 31.2 mg 2,4,6-tripirydyl-S-triazine was dissolved in 1 ml of 40 mM HCl. To prepare 40 mM HCl, commercial standard hydrochloric acid having 12 M concentration was diluted and the desired concentration of hydrochloric acid was obtained, i.e., 40 mM. To make a working solution of 40 mM HCl, 25 µl of 12 M HCl was added to a measuring cylinder and the volume was made up to 10 ml.

A stock solution of $FeCl_3.6H_2O$ having a concentration of 100 mM was prepared by dissolving 0.1 g $FeCl_3.6H_2O$ in 5 ml. The working solution of $FeCl_3.6H_2O$ having 20mM concentration was prepared from the stock solution of 100 mM. To prepare 20 mM $FeCl_3.6H_2O$, a stock solution of $FeCl_3.6H_2O$ having 100 mM concentration was diluted, and the desired concentration of $FeCl_3.6H_2O$ was obtained i.e., 20 mM. To make working solution of 20 mM $FeCl_3.6H_2O$, 1 ml of 100 mM $FeCl_3.6H_2O$ was added to 4 ml of distilled water to obtain the final volume of 5 ml.

4.5 Griess Assay

Griess assay was a colorimetric assay method for measuring total nitrites concentration in the supernatant, with a detection limit of 1.5 µM. Nitrite is an inert oxidized product of nitric oxide, and the physiological storage pool of nitric oxide. Fresh Griess assay reagent mixture was prepared by dissolving 2 ml of Griess reagent, 8 ml deionized water and 5 ml of H_2SO_4. 100 µl plasma sample was added to 130 µl Griess working reagent mixture. Then it was incubated for 30 minutes. Then it was added to a 96-well plate the absorbance was measured using a microplate reader at 548 nm (Brizzolari *et al*., 2021).

Table 4.6 Griess Assay

1.	130 µl Griess assay reagent mixture was added to 100 µl plasma
2.	Incubated the solutions containing the sample for 30 minutes at room temperature

3.	Measured the absorbance at 548 nm

To obtain the standard curve, 1 mM aqueous sodium nitrite solution was used as standard antioxidant solution. Different concentrations of aqueous sodium nitrite solution from 0 µM to 100 µM were prepared using serial dilution. MilliQ water was used for conducting the Griess assay. 600 µl of milliQ water was used as blank.

To prepare a working solution of 1 ml of 1.0 mM nitrite standard solution, a 100 mM nitrite solution was prepared. To obtain 100 mM nitrite solution, 0.0069 g of sodium nitrite was dissolved in 1 ml milliQ water. To obtain 1 mM nitrite solution, 10 µl of 100 mM nitrite solution was added to 990 µl milliQ water.

Table 4.7 Different concentrations of sodium nitrite solution for standard curve

S.No.	Concentration (µM)	Nitrite solution (µl)	MilliQ water (µl)	Final volume (µl)
1.	100	60 µl from stock 1.0 mM solution	540	300
2.	50	300 µl from solution 1	300	300
3.	25	300 µl from solution 2	300	300
4.	12.5	300 µl from solution 3	300	300
5.	6.25	300 µl from solution 4	300	300
6.	3.125	300 µl from solution 5	300	300
7.	1.563	300 µl from solution 6	300	600
8.	0	0	600	600

A freshly prepared Griess working reagent mixture was used every time for conducting the Griess assay. To obtain Griess working reagent mixture following were added:

- 2 ml of Griess reagent,
- 8 ml of milliQ water and
- 5 ml of 1 M H_2SO_4

To prepare 90 ml of 1.0 M H_2SO_4 from concentrated H_2SO_4 (96%, 18M), 85 mL milliQ water was added to an autoclaved 100 ml bottle and 5 ml concentrated H_2SO_4 was added and mixed gently.

4.6 Catalase Activity In-gel Assay

The catalase activity gel was green-blue in colour with light bands where enzyme was present. Catalase enzyme removes the peroxides from the area of the gel it occupies. Removal of peroxide does not allow for the potassium ferricyanide to reduce potassium ferrocyanide which reacts with ferric chloride to form a Prussian blue precipitate. The bands in catalase gels increase with the increase in catalase activity (Weydert *et al*., 2010). This whole experiment was conducted using autoclaved water.

Requirements:

- Running buffer (Tris-glycine-EDTA buffer)
- Gel casting plates
- Gel comb (slot former)
- Loading dye
- Vertical gel electrophoresis apparatus
- DC power supply
- UV-visible transilluminator

Preparing loading gel buffer

- Tris buffer (pH 8.8) – 5 ml

- Glycerol (40%) – 5 ml
- Bromophenol blue (50mg/ml) – 200 µl

Preparing running buffer (Tris-glycine-EDTA buffer)

1. 5x stock solution of running buffer (200 ml) – pH 8.3

- Tris base - 6.06 g
- Glycine – 22.5 g
- EDTA – 0.68 g
- Make up volume with distilled water

2. Working solution of running buffer (300 ml)

- 60 ml of 5x stock solution
- Make up volume with distilled water

Preparation of the 8% native gel for catalase activity assay

To prepare 8% (v/v) native gel, the following components were added:

a. ddH_2O – 4.86 ml
b. Acryl bis – 2.14 ml
c. Tris buffer – 1 ml
d. APS – 120 µl
e. TEMED – 13 µl

Loading and running the gel

1. The glass casting plates were taped at both the ends.
2. The native gel solution was prepared by adding the components stated above.

3. The solution was poured into the taped glass casting plates.

4. The gel comb was inserted after pouring, ensuring no bubbles were trapped alongside the comb and all bubbles were removed before setting of the gel.

5. After the gel got solidified, the gel comb was withdrawn with proper care without disrupting the sample wells.

6. Placed the gel casting plates containing the set gel in the electrophoresis chamber. Added sufficient running buffer to cover the gel until the top of the wells were submerged. Made sure no air pockets were trapped within the wells.

7. Plasma samples were prepared by mixing 19 µl plasma with 6 µl of loading dye.

8. Samples were typically loaded into the wells using a micropipette. Care was taken to prevent the mixing of the samples between wells.

9. The electrodes were connected to a power pack, allowing the electrophoresis apparatus to run at 120 V and 70 mA.

10. Gel casting plates were removed from the electrophoretic unit.

Washing the gel

1. The tape was removed from the gel casting plates, and the gel was placed in a container containing 100 ml of 5% methanol (95 ml water and 5 ml methanol) for 5 minutes without shaking.

2. After 5 minutes, 5% methanol was poured out of the container.

3. Gel was placed in 100 ml autoclaved for 15 minutes.

4. After 15 minutes, autoclaved water was poured out of the container.

5. The gel was incubated in the dark containing autoclaved water and H_2O_2. 100 ml autoclaved water was added to the container having gel followed by adding 120 µl H_2O_2. The gel was incubated for 30 minutes.

6. After 30 minutes, autoclaved water containing H_2O_2 was poured out of the container.

7. 100 ml autoclaved water was poured into the container and the gel was incubated for 5-7 minutes.
8. After 5-7 minutes, autoclaved water was poured out of the container.
9. 2% $FeCl_3.6H_2O$ was prepared by adding 0.6 g $FeCl_3.6H_2O$ in 30 ml autoclaved water. 2% potassium ferricyanide was prepared by adding 0.6 g potassium ferricyanide in 30 ml autoclaved water. Both the solutions were added to an autoclaved bottle, shaken and poured onto the gel.
10. The gel was incubated in the solution for 5-7 minutes.
11. After 5-7 minutes, the solution was poured out of the container.
12. Autoclaved water was poured on the gel.
13. The gel was placed on the UV transilluminator and then photographed. The photographs were later analyzed using Image J software for densitometric analysis.

Protein estimation

Protein estimation of the samples was done to ensure 100 µg of protein in each well. For protein estimation the BSA standard was prepared using the Bradford method.

Requirements:

- BSA (Bovine Serum Albumin) or plasma samples
- Autoclaved water
- Bradford solution

Preparing 5x Bradford solution:

- 100 mg Coomassie brilliant blue
- 47 ml methanol (100%)
- 100 ml Ortho-phosphoric acid (85%)

100 mg Coomassie brilliant blue was dissolved in 47 ml methanol (100%) and then vortexed. Further, 100 ml of ortho-phosphoric acid (85%) was added.

Preparing 1x Bradford solution (40ml):

8 ml of 5x Bradford solution was added to 32 ml autoclaved water to make up the final volume of 40 ml.

Preparing standard curve:

Table 4.8 Different concentrations of BSA for standard curve

S.No.	BSA (µl)	ddH_2O (µl)	Concentration (µg)	Bradford solution (ml)
1.	0	100	Blank	5
2.	10	90	100	5
3.	20	80	200	5
4.	30	70	300	5
5.	40	60	400	5
6.	50	50	500	5

After the standard curve was obtained, protein estimation of plasma samples of COPD patients and healthy controls was carried out in the following manner:

Table 4.9 Protein estimation	
1.	98 µl of autoclaved water was added to 2 µl

2.	5 ml Bradford solution was added
3.	Incubation for 15 minutes at room temperature
4.	Absorbance measured at 595 nm

CHAPTER 5

RESULTS

5. Results

Blood samples were collected from 160 participants. Plasma was isolated within 2-3 hours of collection using centrifugation. No haemolysis was found in any sample, and the supernatant fluid (plasma) was preserved for further assays. Plasma samples were preserved at -20°C.

5.1 Demographics

The study participants were all adults (≥40 years of age), and were smokers or non-smokers. The same plasma blood samples from 160 participants were analysed using DPPH, FRAP and Griess assay.

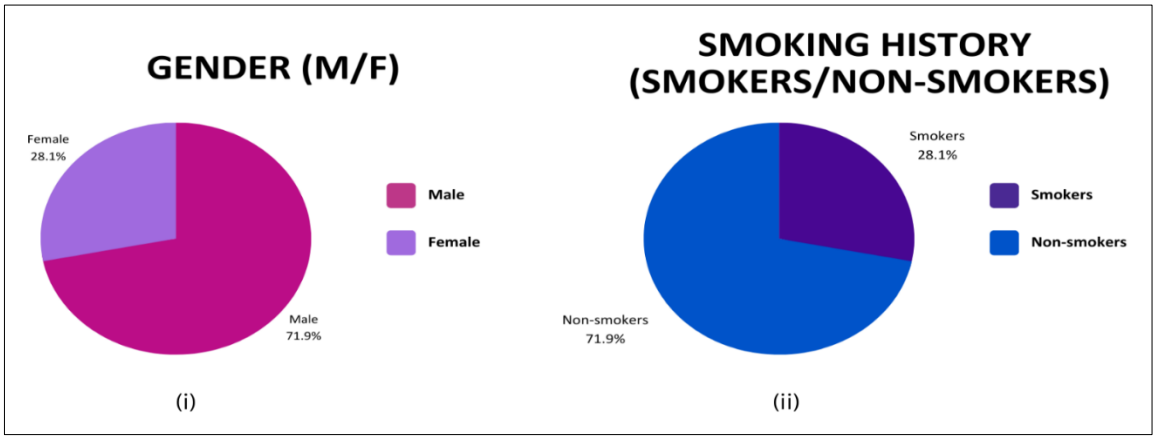


Figure 5.1 the demographics of 160 participants

The participants' baseline characteristics are detailed in Table 5.1. In summary, 28.12% were female and aged in their fifties, and 3.8% were smokers, while 74% were males and aged in their fifties and 52.7% were smokers.

Performa was filled by patients and the following characteristics were noted such as age, sex, smoking history, severity of airflow obstruction (mild, moderate, severe and very severe), CAT score (CAT score less than 10 and CAT score more than 10), GOLD group/ABE classification (GOLD A, GOLD B and GOLD E), and symptoms (breathlessness, cough and mucous).

Table 5.1 Baseline characteristics of participants' (n = 160)	
Age, yrs	54.16 ± 12.49
Sex, M/F	115/45 (71.87%/28.12%)
Smoking history	
Smokers	45 (28.12%)
Non-smokers	115 (71.87%)

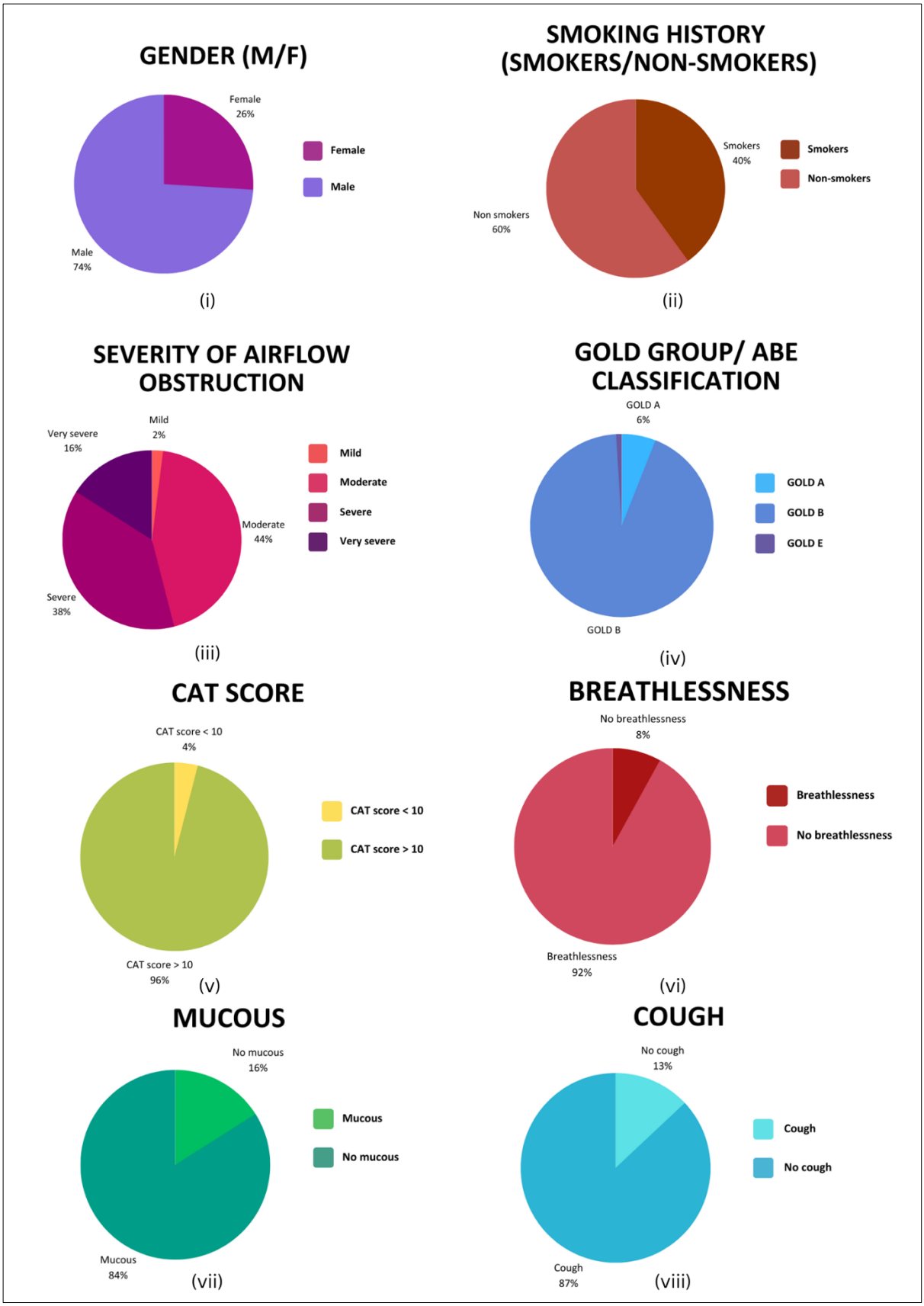


Figure 5.2 shows the demographics of 100 patients based on different parameters (i) Percentage of female and male COPD patients (ii) Percentage of smokers with COPD and non-smokers with COPD (iii) Percentage of mild, moderate, severe and very severe COPD patient based on severity of airflow

obstruction (iv) Percentage of GOLD group A, GOLD group B and GOLD group E based on ABE classification (v) Percentage of COPD patients with CAT score more than 10 and COPD patients with CAT score less than 10 (vi) Percentage of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness (vii) Percentage of COPD patients experiencing mucous production and COPD patients not experiencing mucous production (viii) Percentage of COPD patients experiencing cough and COPD patients not experiencing cough

The patient's baseline characteristics are detailed in Table 5.2. In summary, 26% were female and aged in their fifties and 74% were male and aged in their fifties. Most presented CAT scores of more than 10. Most presented moderate or severe airflow limitation and were classed as GOLD grade B. Most presented breathlessness, mucous and cough.

Table 5.2 Baseline characteristics of patients (n=100)	
Age, yrs	54.48 + 13.37
Sex, M/F	74/26 (74%/26%)
Smoking history	
Smokers	40 (40%)
Non-smokers	60 (60%)
Severity of airflow obstruction	
Mild	2 (2%)
Moderate	44 (44%)
Severe	38 (38%)
Very severe	16 (16%)
CAT score	
CAT score < 10	4 (4%)
CAT score > 10	96 (96%)
GOLD group/ ABE classification	
GOLD A	6 (6%)
GOLD B	93 (93%)
GOLD E	1 (1%)
Symptoms	
Breathlessness	8 (8%)
No breathlessness	92 (92%)
Mucous	16 (16%)

No mucous	84 (84%)
Cough	13 (13%)
No cough	87 (87%)

5.2 DPPH Assay

Total antioxidant capacity (TAC) of COPD patients and healthy controls were measured using the DPPH assay. Unpaired t-test and one-way ANOVA was performed using GraphPad Prism 9 software on data obtained using DPPH assay. LSD test was performed using Costat software to compare the means of concentration (equivalent to ascorbic acid) of COPD patients and healthy controls.

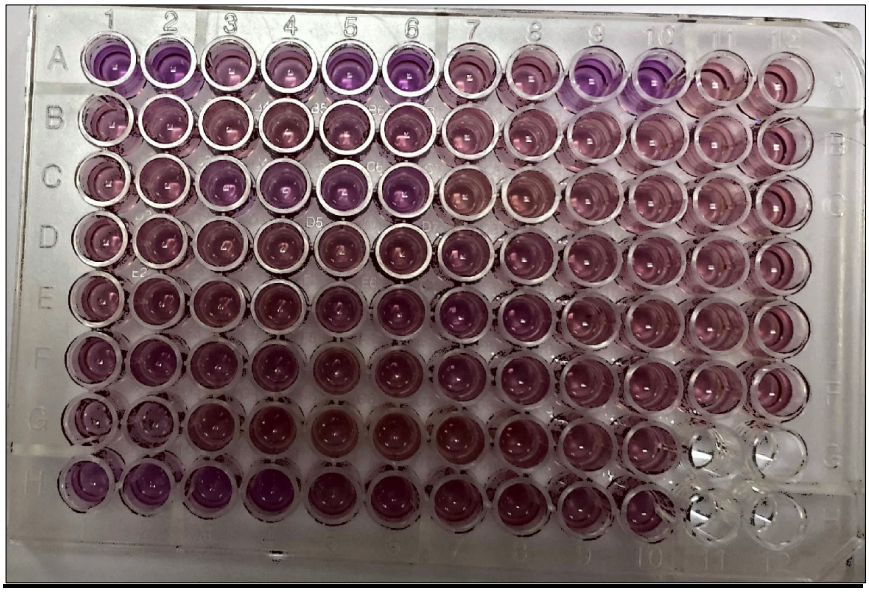


Figure 5.3 ELISA plate used for performing DPPH Assay

In summary, figure 5.3 shows the ELISA plate used for performing DPPH Assay in which wells A1, A2, A5, A6, A9 and A10 contained blank and rest of the wells B2 to H4, B5 to H8, and B9 to F12 contained samples.

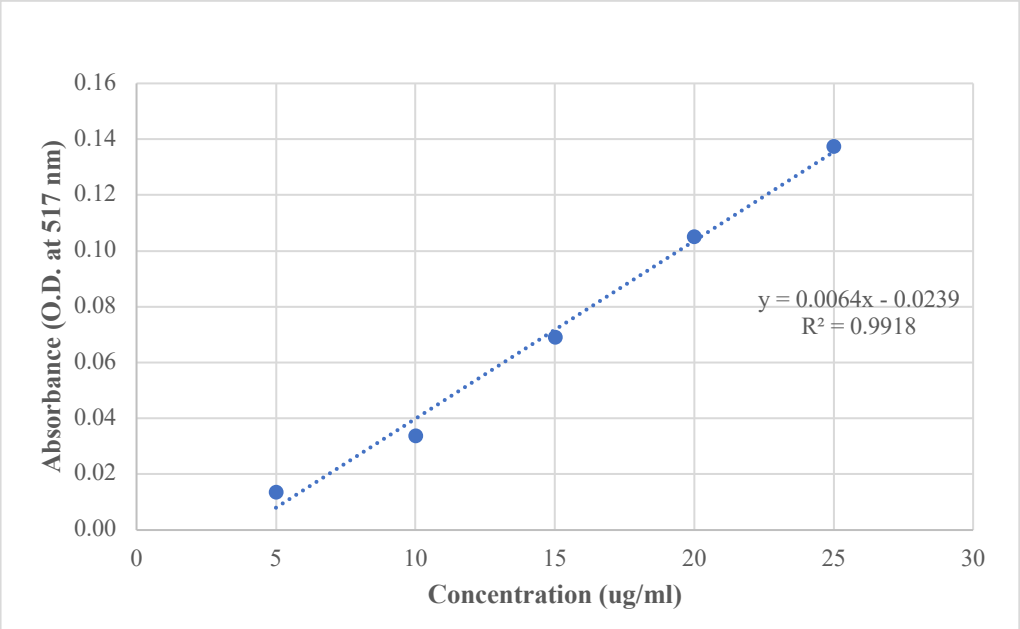


Figure 5.4 Standard curve of Ascorbic Acid for DPPH Assay

The calibration curve is needed to calculate samples concentrations. The concentration of ascorbic acid (µg/ml) was determined using the equation y = 0.0064x + 0.0239 with an R^2 value of 0.9918, where y is the absorbance and x is concentration as shown in the graph (Fig 5.4)

Table 5.3 Ascorbic acid concentrations for standard curve

Ascorbic acid (ug/ml)	Absorbance	DPPH scavenging effect (%) or % Inhibition = [(A_0-A_1) / A_0] × 100	% inhibition
0	0		Blank
5	0.01		2.70
10	0.03		8.11
15	0.07		18.92
20	0.11		27.03
25	0.14		37.84

5.2.1 Comparative analysis of COPD patients and healthy controls

The concentrations (equivalent to ascorbic acid) of patients and healthy controls obtained through the DPPH assay were analyzed using Co Stat software. Compare means test was applied to the data of patients and healthy controls separately.

'Multiple Comparison of Means' tests compare several means and organize the means into subgroups of not-significantly-different means. The significance level is the level of uncertainty (usually 0.05 or 0.01) that the means declared to be different are indeed different. LSD test was performed to compare the means. The test requires a reasonable estimate of the variance (usually from an ANOVA) of the population being tested. The test requires columns of data with the means, the names of the means, and the sample sizes.

Table 5.4 Concentration (along with SD) of patients arranged in descending order

S. No.	*Patient code no.*	*Concentration*
1	8	31.16 a ± 0.42
2	14	23.29 ab ± 0.25
3	9	20.09 abc ± 0.13
4	124	19.67 abc ± 0.39
5	150	19.42 abc ± 0.67
6	1	19.21 abc ± 0.34
7	129	18.73 abc ± 0.21
8	140	18.6 abc ± 0.41
9	21	18.06 abc ± 0.72
10	20	17.82 abc ± 0.16
11	23	16.89 abc ± 0.64
12	61	16.73 abc ± 0.33
13	146	16.44 abc ± 0.19
14	22	16.16 abc ± 0.05
15	147	15.68 abcd ± 0.95
16	6	15.56 abcd ± 0.53
17	63	15.56 abcd ± 0.36
18	7	15.5 abcd ± 0.42
19	19	15.48 abcd ± 0.07
20	138	15.46 abcd ± 0.39
21	65	15.42 abcd ± 0.42
22	42	15.24 abcd ± 0.05
23	64	14.93 bcd ± 0.55
24	69	14.85 bcd ± 0.58

25	122	14.59 bcd ± 0.27
26	5	14.39 bcd ± 0.12
27	25	14.23 bcd ± 0.13
28	2	13.92 bcd ± 0.28
29	126	13.89 bcd ± 0.37
30	136	13.68 bcd ± 0.12
31	37	13.53 bcd ± 0.39
32	66	13.21 bcd ± 0.47
33	131	13.19 bcd ± 0.25
34	70	12.98 bcd ± 0.24
35	71	12.67 bcd ± 0.55
36	98	12.61 bcd ± 0.38
37	72	12.43 bcd ± 0.86
38	40	12.43 bcd ± 0.05
39	67	12.43 bcd ± 0.07
40	92	12.35 bcd ± 0.16
41	62	12.35 bcd ± 0.63
42	59	12.28 bcd ± 0.32
43	43	12.12 bcd ± 0.49
44	39	11.81 bcd ± 0.16
45	143	11.81 bcd ± 0.16
46	80	11.69 bcd ± 0.18
47	78	11.65 bcd ± 0.09
48	85	11.65 bcd ± 0.18
49	50	11.57 bcd ± 0.27
50	141	11.52 bcd ± 0.34
51	56	11.4 bcd ± 0.09
52	84	11.16 bcd ± 0.07
53	76	11.1 bcd ± 0.27
54	75	11.1 bcd ± 0.08
55	31	11.03 bcd ± 0.05
56	93	10.87 bcd ± 0.22

57	81	10.58 bcd ± 0.05
58	26	10.4 bcd ± 0.35
59	108	10.2 bcd ± 0.16
60	100	10.14 bcd ± 0.06
61	97	9.98 bcd ± 0.47
62	96	9.98 bcd ± 0.22
63	30	9.93 bcd ± 0.50
64	29	9.78 bcd ± 0.06
65	48	9.7 bcd ± 0.02
66	110	9.69 bcd ± 0.31
67	68	9.62 bcd ± 0.58
68	117	9.59 bcd ± 0.18
69	87	9.56 bcd ± 0.29
70	28	9.41 bcd ± 0.30
71	125	9.34 bcd ± 0.95
72	86	9.31 bcd ± 0.31
73	94	9.26 bcd ± 0.36
74	101	9.12 bcd ± 0.00
75	149	8.78 bcd ± 0.00
76	88	8.6 bcd ± 0.23
77	148	8.58 bcd ± 0.31
78	57	8.53 bcd ± 0.19
79	134	8.42 bcd ± 0.30
80	38	7.98 bcd ± 0.16
81	33	7.9 bcd ± 0.30
82	77	7.74 bcd ± 0.13
83	123	7.64 bcd ± 0.05
84	99	7.62 bcd ± 0.10
85	154	7.58 bcd ± 0.13
86	104	7.53 bcd ± 0.32
87	90	7.43 bcd ± 0.27
88	137	7.17 cd ± 0.35

89	144	6.89 cd ± 0.41
90	156	6.63 cd ± 0.28
91	95	6.23 cd ± 0.55
92	107	6.03 cd ± 0.78
93	127	5.59 cd ± 0.47
94	116	5.55 cd ± 0.77
95	113	5.3 cd ± 0.41
96	155	5.3 cd ± 0.63
97	103	5.14 cd ± 0.30
98	132	4.01 cd ± 0.32
99	118	0 d ± 0.00
100	119	0 d ± 0.00

In summary, the concentration of patients obtained using the DPPH assay ranged from 31.16 to 0. The highest concentration was shown by patient number 8 viz., 31.16 ± 0.42. The lowest concentration was shown by patients number 118 and 119 viz., 0 ± 0.00.

Table 5.5 Concentration (along with SD) of controls arranged in descending order		
S. No.	*Control code no.*	*Concentration*
1	52	17.57 a ± 0.72
2	109	16.65 ab ± 0.72
3	79	16.63 ab ± 0.72
4	105	16.52 ab ± 0.14
5	53	15.87 ab ± 0.25
6	112	13.99 abc ± 0.64
7	178	13.66 abc ± 0.10
8	176	13.34 abc ± 0.15
9	55	12.95 abc ± 0.30
10	114	12.74 abc ± 0.32
11	186	12.74 abc ± 0.03
12	24	12.64 abc ± 0.30
13	73	12.6 abc ± 0.23

14	188	12.59 abc ± 0.06
15	35	12.56 abc ± 0.31
16	111	12.56 abc ± 0.16
17	182	12.51 abc ± 0.28
18	106	12.51 abc ± 0.38
19	120	12.49 abc ± 0.24
20	13	12.41 abc ± 0.07
21	15	12.38 abc ± 0.33
22	102	12.33 abc ± 0.41
23	16	12.33 abc ± 0.54
24	4	12.23 abc ± 0.47
25	3	11.97 abc ± 0.35
26	51	11.81 abc ± 0.07
27	175	11.73 abc ± 0.37
28	31	11.63 abc ± 0.27
29	196	11.6 abc ± 0.35
30	82	11.41 abc ± 0.34
31	174	11.36 abc ± 0.24
32	135	11.08 abc ± 0.70
33	145	10.63 abc ± 0.39
34	181	10.61 abc ± 0.09
35	183	10.55 abc ± 0.30
36	185	10.53 abc ± 0.39
37	152	10.47 abc ± 0.25
38	130	10.45 abc ± 0.00
39	121	10.3 abc ± 0.31
40	184	9.91 abc ± 0.47
41	17	9.83 abc ± 0.31
42	187	9.78 abc ± 0.33
43	179	9.75 abc ± 0.16
44	18	9.67 abc ± 0.23
45	180	9.56 abc ± 0.86

46	191	9.52 abc ± 0.62
47	32	9.52 abc ± 0.03
48	169	8.89 abc ± 0.32
49	189	8.89 abc ± 0.33
50	153	8.68 abc ± 0.27
51	190	8.42 abc ± 0.03
52	139	8.36 abc ± 0.43
53	34	7.72 bc ± 0.19
54	192	7.51 bc ± 0.36
55	177	7.35 bc ± 0.05
56	36	7.09 bc ± 0.32
57	45	5.77 c ± 0.49
58	27	5.3 c ± 0.19
59	41	4.81 c ± 0.30
60	196	4.67 c ± 0.43

In summary, the concentration of healthy controls obtained using the DPPH assay ranged from 17.57 to 4.67. The highest concentration was shown by control number 52 viz., 17.57 ± 0.72. The lowest concentration was shown by control number 196 viz., 4.67 ± 0.43.

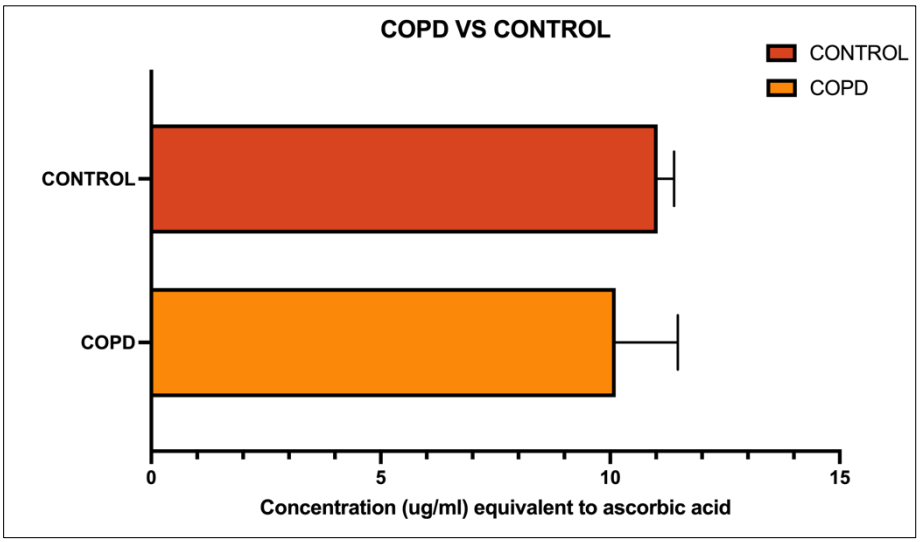


Figure 5.5 Total antioxidant capacity (TAC) of plasma of healthy controls and COPD patients

In summary, figure 5.5 shows the mean of antioxidant capacity of the plasma obtained from the COPD patients corresponded to 10.12 ± 0.04 μg/ml of ascorbic acid equivalent. The mean of antioxidant capacity obtained from control plasma corresponded to 11.03 ± 0.02 μg/ml of ascorbic acid equivalent.

Table 5.6 Statistical analysis using unpaired t-test of 100 COPD patients and 60 healthy control.	
Column B	**CONTROL**
vs.	vs.
Column A	**COPD**
Unpaired t test	
P value	0.6075
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.5146, df=158
How big is the difference?	
Mean of column A	10.12
Mean of column B	11.03
Difference between means (B - A) ± SEM	0.9099 ± 1.768
95% confidence interval	-2.582 to 4.402
R squared (eta squared)	0.001674
F test to compare variances	
F, DFn, Dfd	23.55, 99, 59
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes

Data analyzed	
Sample size, column A	100
Sample size, column B	60

In summary, table 5.6 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma of COPD patients and healthy controls. The mean concentration equivalent to ascorbic acid for COPD patients and healthy controls were 10.12 ± 0.04 μg/ml and 11.03 ± 0.02 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from healthy volunteers and patients with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.2 Comparative analysis of male and female COPD patients

Table 5.7 Statistical analysis using unpaired t-test of female and male COPD patients	
Table Analyzed	Col: Unpaired t test - male vs female
Column B	Male
vs.	vs.
Column A	Female
Unpaired t test	
P value	0.5649
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.5775, df=98
How big is the difference?	
Mean of column A	11.44

Mean of column B	9.658
Difference between means (B - A) ± SEM	-1.784 ± 3.090
95% confidence interval	-7.916 to 4.347
R squared (eta squared)	0.003392
F test to compare variances	
F, DFn, Dfd	15.22, 73, 25
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	26
Sample size, column B	74

In summary, table 5.7 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from the female COPD patients and male patients with COPD. The mean concentration (equivalent to ascorbic acid) for female COPD patients and male patients with COPD were 9.658 ± 0.32 μg/ml and 11.44 ± 0.04 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from female COPD patients and male patients with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

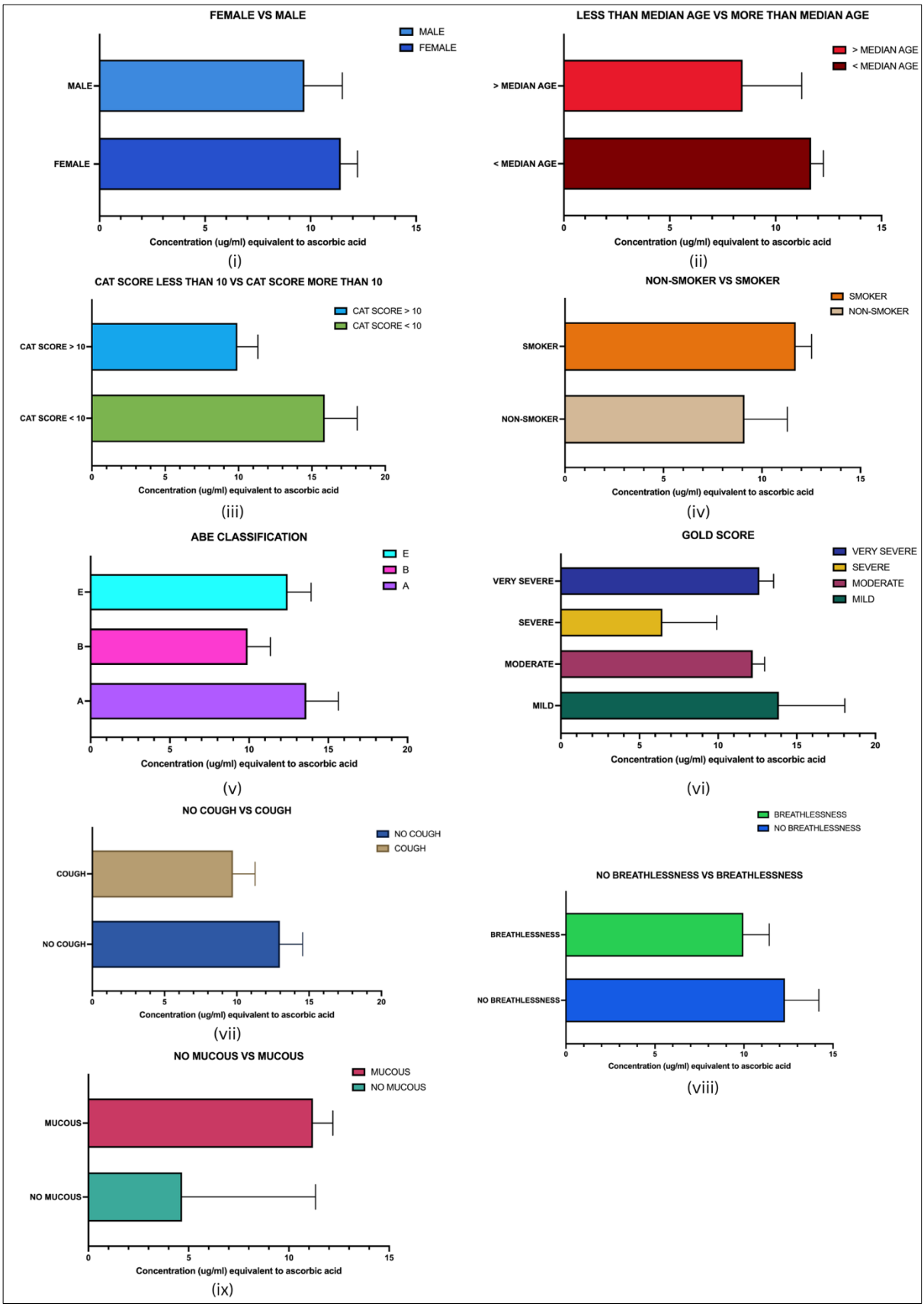


Figure 5.6 Total antioxidant capacity (TAC) of plasma of COPD patients based on different parameters (i) Total antioxidant capacity (TAC) of plasma of female and male COPD patients (ii) Total antioxidant capacity (TAC) of plasma of COPD patients with age less than median age and with age more than median

age (iii) Total antioxidant capacity (TAC) of plasma of COPD patients with CAT score more than 10 and CAT score less than 10 (iv) Total antioxidant capacity (TAC) of plasma of smokers with COPD and non-smokers with COPD (v) Total antioxidant capacity (TAC) of plasma of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification (vi) Total antioxidant capacity (TAC) of plasma of COPD patients classified as mild, moderate, severe and very severe based on GOLD score (vii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing cough and COPD patients not experiencing cough (viii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness (ix) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

5.2.3 Comparative analysis of COPD patients on the basis of age: COPD patients having age less than median age (median age = 58) and COPD patients having age more than median age

Table 5.8 Statistical analysis using unpaired t-test of COPD patients with age less than median age and with age more than median age.

Table Analyzed	Col: Unpaired t test - <median age vs >median age
Column B	> median age
vs.	vs.
Column A	< median age
Unpaired t test	
P value	0.2245
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.222, df=98
How big is the difference?	
Mean of column A	11.67
Mean of column B	8.373

Difference between means (B - A) ± SEM	-3.300 ± 2.700
95% confidence interval	-8.657 to 2.058
R squared (eta squared)	0.01502
F test to compare variances	
F, DFn, Dfd	20.13, 46, 52
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	53
Sample size, column B	47

In summary, table 5.8 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients with age less than median age and with age more than median age. The mean concentration (equivalent to ascorbic acid) for COPD patients with age less than median age and with age more than median age were 11.67 ± 0.32 μg/ml and 8.37 ± 0.33 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients with age less than median age and with age more than median age did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.4 Comparative analysis of COPD patients on the basis of CAT score: COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10

Table 5.9 Statistical analysis using unpaired t-test of COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10	
Table Analyzed	Col: Unpaired t test - CAT score <10 vs CAT score >10

Column B	CAT SCORE >10
vs.	vs.
Column A	CAT SCORE <10
Unpaired t test	
P value	0.3940
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8561, df=98
How big is the difference?	
Mean of column A	15.80
Mean of column B	9.886
Difference between means (B - A) ± SEM	-5.909 ± 6.902
95% confidence interval	-19.61 to 7.788
R squared (eta squared)	0.007424
F test to compare variances	
F, DFn, Dfd	9.190, 95, 3
P value	0.0905
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	96

In summary, table 5.9 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10. The mean concentration

(equivalent to ascorbic acid) for COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10 were 15.80 ± 0.44 μg/ml and 9.89 ± 0.32 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10 did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.5 Comparative analysis of smoker and non-smoker COPD patients

Table 5.10 Statistical analysis using unpaired t-test of non-smokers with COPD and smokers with COPD

Table Analyzed	Col: Unpaired t test – non-smokers vs smokers
Column B	Smokers
vs.	vs.
Column A	Non-smokers
Unpaired t test	
P value	0.3545
P value summary	Ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9303, df=98
How big is the difference?	
Mean of column A	9.096
Mean of column B	11.66
Difference between means (B - A) ± SEM	2.567 ± 2.759
95% confidence interval	-2.908 to 8.042
R squared (eta squared)	0.008754

F test to compare variances	
F, DFn, Dfd	11.09, 59, 39
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	60
Sample size, column B	40

In summary, table 5.10 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from non-smoker COPD patients and smokers with COPD. The mean concentration (equivalent to ascorbic acid) for non-smoker COPD patients and smokers with COPD were 9.096 ± 0.32 μg/ml and 11.66 ± 0.33 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.6 Comparative analysis of COPD patients on the basis of ABE classification: COPD patients classified as group A, B and E

Table 5.11 Statistical analysis using one-way ANOVA of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification

Table Analyzed	Col: One-way ANOVA - ABE classification				
Data sets analyzed	A-C				
ANOVA summary					
F	0.2269				

P value	0.7974				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.004657				
Brown-Forsythe test					
F (DFn, DFd)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	84.12	2	42.06	F (2, 97) = 0.2269	P=0.7974
Residual (within columns)	17978	97	185.3		
Total	18062	99			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	100				

In summary, table 5.11 shows analysis of one-way ANOVA performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification. The mean concentration (equivalent to ascorbic acid) of COPD patients classified as GOLD group A, GOLD group B and GOLD group E were 13.61 ± 0.40 μg/ml, 9.91 ± 0.32 μg/ml and 12.43 ± 0.58 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients classified as GOLD group A, GOLD group B and GOLD group E did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.7 Comparative analysis of COPD patients on the basis of GOLD score: COPD patients classified as mild, moderate, severe and very severe

Table 5.12 Statistical analysis using one-way ANOVA of COPD patients classified as mild, moderate, severe and very severe based on GOLD score

Table Analyzed	Col: One-way ANOVA - GOLD score				
Data sets analyzed	A-D				
ANOVA summary					
F	1.564				
P value	0.2032				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.04659				
Brown-Forsythe test					
F (DFn, DFd)	0.6765 (3, 96)				
P value	0.5685				

P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	841.5	3	280.5	F (3, 96) = 1.564	P=0.2032
Residual (within columns)	17221	96	179.4		
Total	18062	99			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	100				

In summary, table 5.12 shows analysis of one-way ANOVA performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients classified as mild, moderate, severe and very severe based on GOLD score. The mean concentration (equivalent to ascorbic acid) for COPD patients classified as mild, moderate, severe and very severe were 13.86 ± 0.47 μg/ml, 12.20 ± 0.33 μg/ml, 6.46 ± 0.31 μg/ml and 12.62 ± 0.32 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.8 Comparative analysis of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness

Table 5.13 Statistical analysis using unpaired t-test of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness	
Table Analyzed	Col: Unpaired t test – non breathlessness vs breathlessness
Column B	Breathlessness
vs.	vs.
Column A	Non-breathlessness
Unpaired t test	
P value	0.6307
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.4823, df=98
How big is the difference?	
Mean of column A	12.34
Mean of column B	9.929
Difference between means (B - A) ± SEM	-2.411 ± 4.998
95% confidence interval	-12.33 to 7.508
R squared (eta squared)	0.002368
F test to compare variances	
F, DFn, Dfd	6.831, 91, 7
P value	0.0119
P value summary	*
Significantly different (P < 0.05)?	Yes

Data analyzed	
Sample size, column A	8
Sample size, column B	92

In summary, table 5.13 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness were 9.93 ± 0.33 μg/ml and 12.34 ± 0.25 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.9 Comparative analysis of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

Table 5.14 Statistical analysis using one-way ANOVA of COPD patients experiencing mucous and COPD patients not experiencing mucous	
Table Analyzed	Col: Unpaired t test - no mucous vs mucous
Column B	Mucous
vs.	vs.
Column A	No mucous
Unpaired t test	
P value	0.0781
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.781, df=98

How big is the difference?	
Mean of column A	4.671
Mean of column B	11.16
Difference between means (B - A) ± SEM	6.490 ± 3.645
95% confidence interval	-0.7428 to 13.72
R squared (eta squared)	0.03134
F test to compare variances	
F, DFn, Dfd	8.574, 15, 83
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	16
Sample size, column B	84

In summary, table 5.14 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing mucous production and COPD patients not experiencing mucous were 11.19 ± 0.31 μg/ml and 4.66 ± 0.38 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.2.10 Comparative analysis of COPD patients experiencing cough and COPD patients not experiencing cough

Table 5.15 Statistical analysis using unpaired t-test of COPD patients experiencing cough and COPD patients not experiencing cough	
Table Analyzed	Col: Unpaired t test
Column B	Cough
vs.	vs.
Column A	No cough
Unpaired t test	
P value	0.4210
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8081, df=98
How big is the difference?	
Mean of column A	12.95
Mean of column B	9.700
Difference between means (B - A) ± SEM	-3.251 ± 4.023
95% confidence interval	-11.24 to 4.733
R squared (eta squared)	0.006619
F test to compare variances	
F, DFn, Dfd	6.225, 86, 12
P value	0.0012
P value summary	**
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	13
Sample size, column B	87

In summary, table 5.15 shows analysis of unpaired t-test performed on the data obtained from 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing cough and COPD patients not experiencing cough. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing cough and COPD patients not experiencing cough were 9.73 ± 0.31 μg/ml and 12.97 ± 0.33 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from COPD patients experiencing cough and COPD patients not experiencing cough did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3 FRAP Assay

Total antioxidant capacity (TAC) of COPD patients and healthy controls were measured using the FRAP assay. Unpaired t-test and one-way ANOVA was performed using GraphPad Prism 9 software on data obtained using FRAP assay. LSD test was performed using Co Stat software to compare the means of concentration (equivalent to ascorbic acid) of COPD patients and healthy controls.

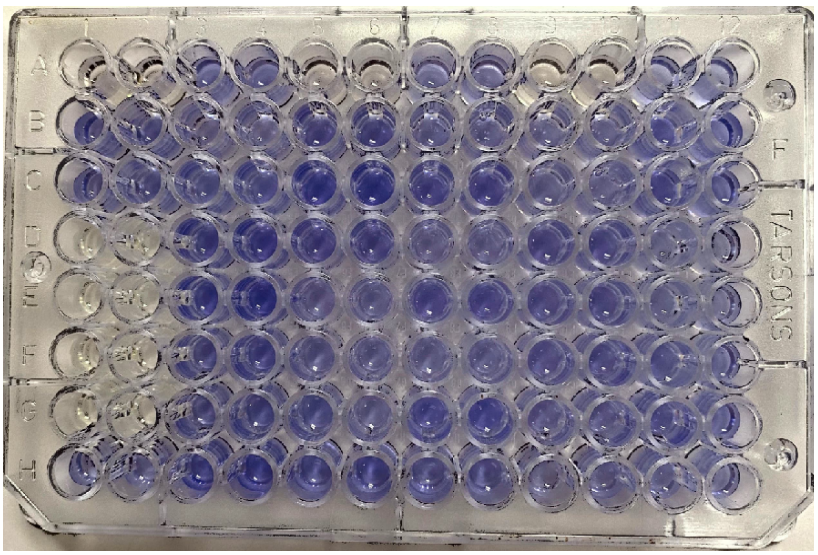


Figure 5.7 ELISA plate used for performing FRAP Assay

Figure 5.7 shows the ELISA plate used for performing FRAP Assay in which wells A1, A2, A5, A6, A9 and A10 contained blank, wells B1-B2 to H1-H2 contained different concentration of ascorbic acid for plotting standard curve and rest of the wells A3 to H4, B5 to H8, and B9 to H12 contained samples.

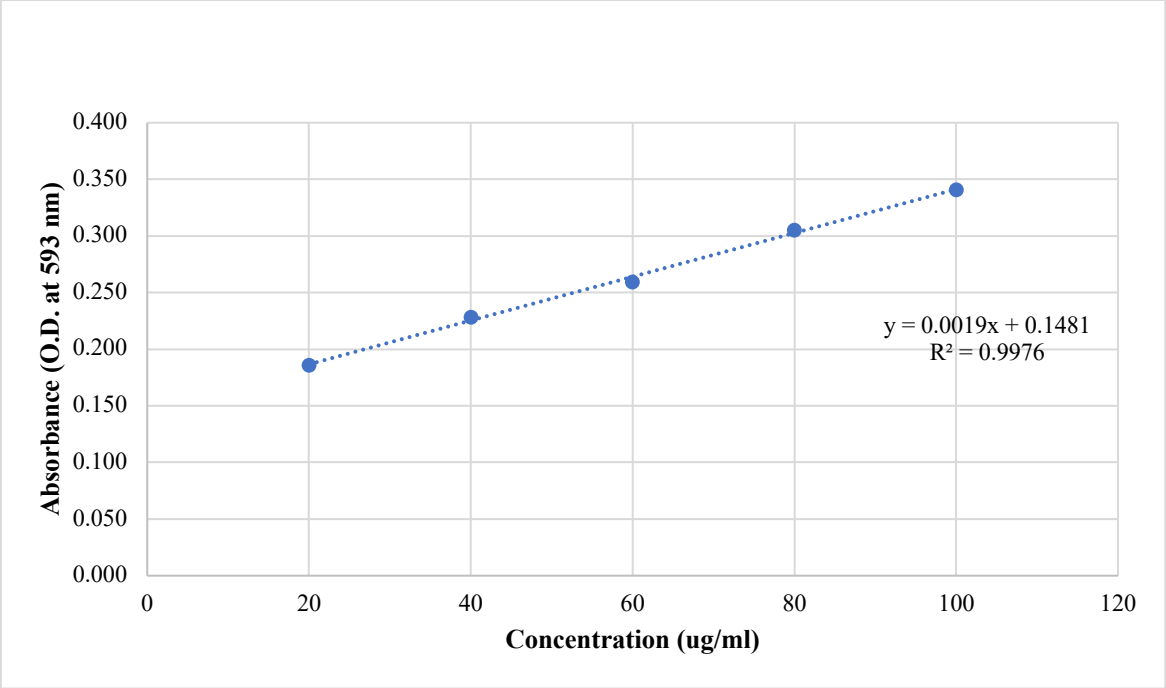


Figure 5.8 Standard curve of Ascorbic Acid for FRAP Assay

The calibration curve is needed to calculate samples concentrations. The concentration of ascorbic acid (µg/ml) was determined using the equation y = 0.0064x + 0.0239 with an R^2 value of 0.9918, where y is the absorbance and x is concentration as shown in the graph (Fig 5.8)

Table 5.16 Ascorbic acid concentrations for standard curve

Ascorbic acid (ug/ml)	Absorbance (593 nm)
0	0.107
20	0.186
40	0.229
60	0.260
80	0.305
100	0.341

5.3.1 Comparative Analysis of COPD Patients and healthy controls

The concentrations (equivalent to ascorbic acid) of patients and healthy controls obtained through the FRAP assay were analyzed using Co Stat software. Compare means test was applied to the data of patients and healthy controls separately. 'Multiple Comparison of Means' tests compare several means and organize the means into subgroups of not-significantly-different means. The significance level is the level of uncertainty (usually 0.05 or 0.01) that the means declared to be different are indeed different.

LSD test was performed to compare the means. The test requires a reasonable estimate of the variance (usually from an ANOVA) of the population being tested. The test requires columns of data with the means, the names of the means, and the sample sizes.

Table 5.17 Concentration (along with SD) of controls arranged in descending order

S. No.	*Patient code no.*	*Concentration*
1	78	317.16 a ± 0.21
2	70	299.14 ab ± 0.46
3	81	277.53 abc ± 0.11
4	21	262.9 abcd ± 0.50
5	67	259.51 abcde ± 0.43
6	31	242.74 abcdef ± 0.14
7	72	197.32 abcdef ± 0.41
8	25	181.68 abcdef ± 0.24
9	63	161.93 abcdef ± 0.34
10	69	160.42 abcdef ± 0.30
11	97	155.65 abcdef ± 0.53
12	38	145.67 abcdef ± 0.19
13	62	139.19 abcdef ± 0.35
14	154	135.5 abcdef ± 0.26
15	61	122.3 abcdef ± 0.20
16	98	121.53 abcdef ± 0.34
17	64	111.33 abcdef ± 0.47

18	95	101.17 abcdef ± 0.76
19	37	92.37 abcdef ± 0.73
20	146	91.42 abcdef ± 0.34
21	42	83.68 abcdef ± 0.58
22	148	78.81 abcdef ± 0.65
23	103	78.21 abcdef ± 0.89
24	140	75.21 abcdef ± 0.53
25	141	73.47 abcdef ± 0.09
26	138	70.47 abcdef ± 0.29
27	84	64.4 abcdef ± 0.22
28	76	63.69 abcdef ± 0.77
29	30	63.48 abcdef ± 0.50
30	26	62.18 bcdef ± 0.21
31	86	59.24 bcdef ± 0.43
32	80	58.4 bcdef ± 0.28
33	93	55.4 bcdef ± 0.33
34	65	53.63 bcdef ± 0.26
35	104	52.49 bcdef ± 0.37
36	150	48.72 bcdef ± 0.64
37	50	48.69 bcdef ± 0.09
38	75	48.32 bcdef ± 0.22
39	92	48.28 bcdef ± 0.31
40	77	46.6 bcdef ± 0.50
41	94	44.61 cdef ± 0.98
42	39	44.13 cdef ± 0.23
43	40	43.53 cdef ± 0.21
44	85	43.09 cdef ± 0.23
45	132	42.75 cdef ± 0.20
46	155	41.63 cdef ± 0.62
47	7	39.4 cdef ± 0.64
48	43	33.6 cdef ± 0.61
49	107	32.9 cdef ± 1.63

50	23	31.63 cdef ± 0.31
51	56	31.42 cdef ± 0.25
52	147	29.75 cdef ± 0.28
53	68	28.61 cdef ± 0.20
54	116	27.59 cdef ± 0.21
55	59	25.23 cdef ± 0.13
56	87	25.03 cdef ± 0.11
57	149	23.37 def ± 0.13
58	134	21.35 def ± 0.68
59	99	21.16 def ± 0.13
60	144	20.14 def ± 0.20
61	28	19.44 def ± 0.27
62	33	18.21 def ± 0.62
63	6	16.63 def ± 0.00
64	143	16.37 def ± 0.20
65	88	16.09 def ± 0.26
66	22	15.39 def ± 0.99
67	90	14.4 def ± 0.18
68	1	13.86 def ± 0.00
69	2	13.84 def ± 0.62
70	137	11.54 def ± 0.34
71	96	10.7 def ± 0.41
72	100	10.47 def ± 0.30
73	71	10.25 def ± 0.31
74	136	9.67 def ± 0.13
75	57	8.74 ef ± 0.30
76	48	8.65 ef ± 0.37
77	66	7.54 ef ± 0.11
78	110	5.63 ef ± 0.34
79	5	4.67 f ± 0.14
80	117	4.33 f ± 0.05
81	8	4.09 f ± 0.23

82	113	0.63 f ± 0.17
83	156	0 f ± 0.05
84	118	0 f ± 0.00
85	119	0 f ± 0.00
86	108	0 f ± 0.00
87	9	0 f ± 0.00
88	29	0 f ± 0.00
89	101	0 f ± 0.00
90	20	0 f ± 0.00
91	131	0 f ± 0.00
92	14	0 f ± 0.00
93	19	0 f ± 0.00
94	122	0 f ± 0.00
95	123	0 f ± 0.00
96	124	0 f ± 0.00
97	125	0 f ± 0.00
98	126	0 f ± 0.00
99	127	0 f ± 0.00
100	129	0 f ± 0.00

In summary, the concentration in patients obtained using the FRAP assay ranged from 317.16 to 0. The highest concentration was shown by patient number 78 viz., 317.16 ± 0.21. The lowest concentration was shown by patients number 9, 14, 19, 20, 29, 101, 108, 118, 119, 122, 123, 124, 125, 126, 127, 129, 131, 156 viz., 0 ± 0.00.

Table 5.18 Concentration (along with SD) of controls arranged in descending order		
S. No.	*Control code no.*	*Concentration*
1	45	164.49 a ± 0.21
2	34	79.86 a ± 0.46
3	52	65.24 a ± 0.11
4	3	63.98 a ± 0.50
5	35	63.4 a ± 0.43
6	24	58.89 a ± 0.14

7	152	53.63 a ± 0.00
8	32	48.28 a ± 0.41
9	51	46.95 a ± 0.00
10	183	44.19 a ± 0.24
11	79	39.26 a ± 0.00
12	36	37.42 a ± 0.34
13	120	36.75 a ± 0.00
14	153	36.75 a ± 0.30
15	182	32.58 a ± 0.53
16	73	32.58 a ± 0.19
17	109	32.58 a ± 0.00
18	4	32.49 a ± 0.35
19	41	32.4 a ± 0.26
20	105	29.41 a ± 0.20
21	53	29.41 a ± 0.34
22	121	29.4 a ± 0.47
23	186	27.41 a ± 0.76
24	18	27.32 a ± 0.73
25	196	23.14 a ± 0.34
26	180	23.14 a ± 0.58
27	130	20.3 a ± 0.65
28	112	20.3 a ± 0.89
29	176	18.21 a ± 0.53
30	185	16.79 a ± 0.09
31	188	16.46 a ± 0.29
32	111	16.46 a ± 0.22
33	175	15.37 a ± 0.77
34	178	13.62 a ± 0.50
35	181	13.28 a ± 0.21
36	82	11.53 a ± 0.43
37	177	11.53 a ± 0.28
38	139	11.44 a ± 0.33

39	27	6.35 a ± 0.26
40	191	6.26 a ± 0.37
41	31	6.26 a ± 0.64
42	135	6.26 a ± 0.09
43	192	2.26 a ± 0.22
44	114	1 a ± 0.31
45	179	1 a ± 0.50
46	189	0 a ± 0.00
47	190	0 a ± 0.00
48	187	0 a ± 0.00
49	184	0 a ± 0.00
50	145	0 a ± 0.00
51	17	0 a ± 0.00
52	16	0 a ± 0.00
53	55	0 a ± 0.00
54	174	0 a ± 0.00
55	83	0 a ± 0.00
56	15	0 a ± 0.00
57	102	0 a ± 0.00
58	106	0 a ± 0.00
59	13	0 a ± 0.00
60	169	0 a ± 0.00

In summary, the concentration in healthy controls obtained using the FRAP assay ranged from 164.49 to 0. The highest concentration was shown by control number 45 viz., 164.49 ± 0.21. The lowest concentration was shown by control number 13, 15, 16, 17, 55, 83, 102, 106, 145, 169, 174, 184, 187, 189, 190 viz., 0 ± 0.00.

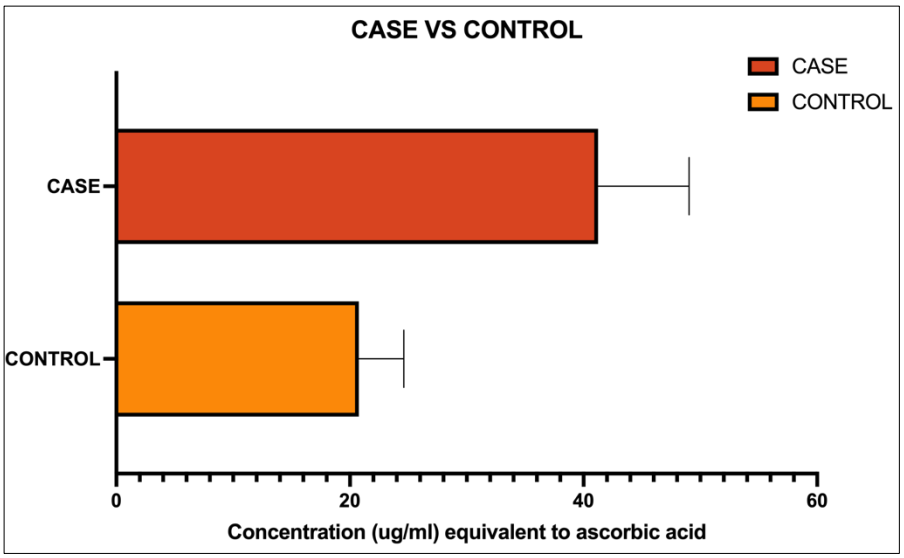


Figure 5.9 Total antioxidant capacity (TAC) of plasma of healthy controls and COPD patients

In summary, figure 5.9 shows the mean of antioxidant capacity of the plasma obtained from the COPD patients corresponded to 57.02 ± 0.27 µg/ml of ascorbic acid equivalent. The mean of antioxidant capacity obtained from control plasma corresponded to 23.43 ± 0.22 µg/ml of ascorbic acid equivalent. The antioxidant capacity of plasma obtained from healthy volunteers and patients with COPD showed significant difference ($p < 0.05$).

Table 5.19 Statistical analysis using unpaired t-test of 100 COPD patients and 60 healthy control.	
Table Analyzed	Col: Unpaired t test - COPD VS CONTROL
Column B	CONTROL
vs.	vs.
Column A	COPD
Unpaired t test	
P value	0.0007
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.446, df=158

How big is the difference?	
Mean of column A	57.02
Mean of column B	23.43
Difference between means (B - A) ± SEM	-33.59 ± 9.748
95% confidence interval	-52.85 to -14.34
R squared (eta squared)	0.06990
F test to compare variances	
F, DFn, Dfd	6.871, 99, 59
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	100
Sample size, column B	60

In summary, table 5.19 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma of COPD patients and healthy controls. The mean concentration equivalent to ascorbic acid for COPD patients and healthy controls were 57.02 ± 0.04 μg/ml and 23.43 ± 0.02 μg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from healthy volunteers and patients with COPD showed significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3.2 Comparative Analysis of Male and Female COPD Patients

Table 5.20 Statistical analysis using unpaired t-test of female and male COPD patients	
Table Analyzed	Col: Unpaired t test - MALE VS FEMALE
Column B	Female

vs.	vs.
Column A	Male
Unpaired t test	
P value	0.4547
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.7507, df=98
How big is the difference?	
Mean of column A	60.25
Mean of column B	47.84
Difference between means (B - A) ± SEM	-12.41 ± 16.53
95% confidence interval	-45.21 to 20.39
R squared (eta squared)	0.005717
F test to compare variances	
F, DFn, Dfd	1.208, 73, 25
P value	0.6088
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	74
Sample size, column B	26

In summary, table 5.20 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from the female COPD patients and male patients with COPD. The mean concentration (equivalent to ascorbic acid) for female COPD patients and male patients with COPD were 47.84 ± 0.32 µg/ml and 60.25 ± 0.04 µg/ml respectively using FRAP assay. The

antioxidant capacity of plasma obtained from female COPD patients and male patients with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

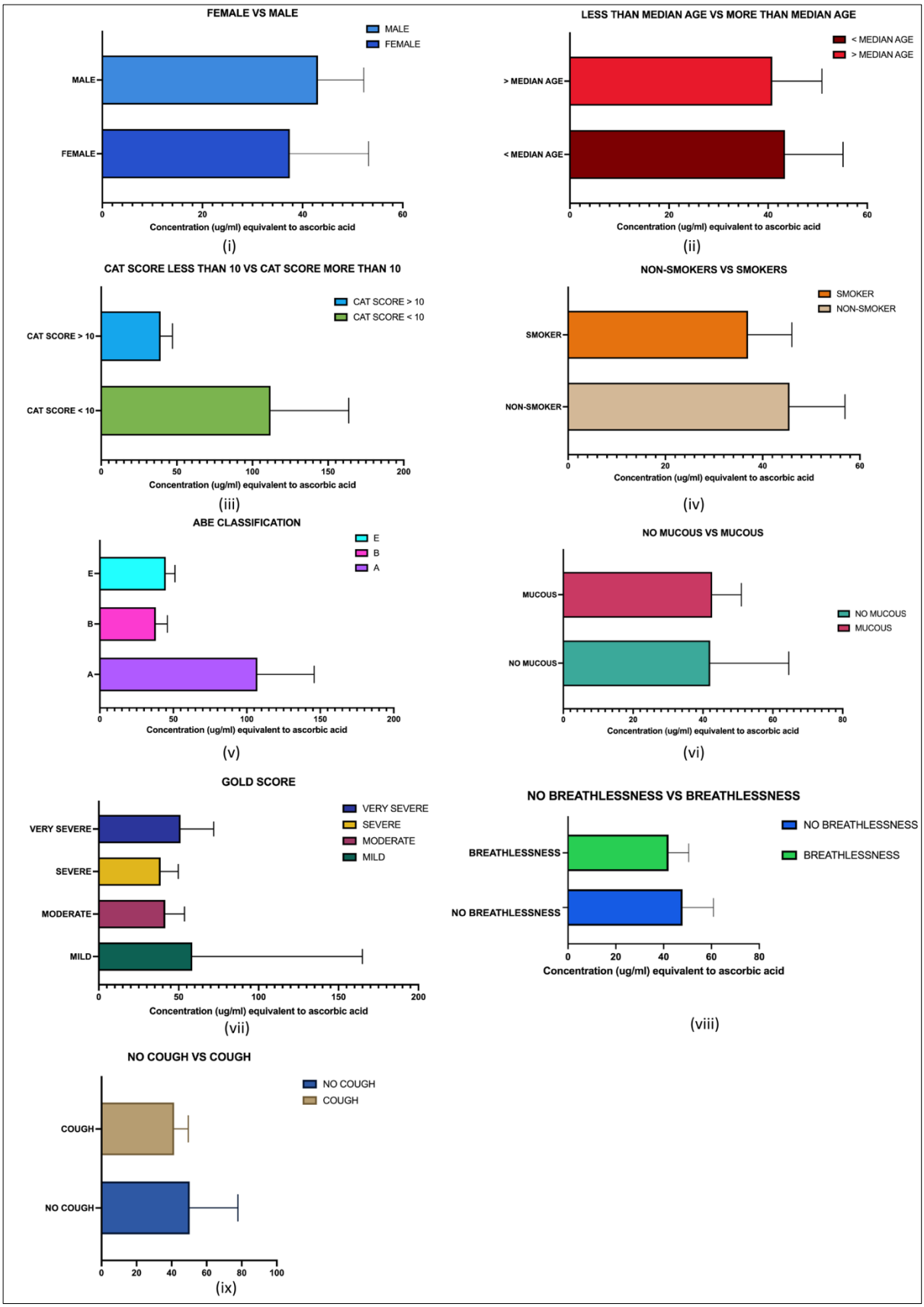


Figure 5.10 Total antioxidant capacity (TAC) of plasma of COPD patients based on different parameters (i) Total antioxidant capacity (TAC) of plasma of female and male COPD patients (ii) Total antioxidant capacity (TAC) of plasma of COPD patients with age less than median age and with age more than median

age (iii) Total antioxidant capacity (TAC) of plasma of COPD patients with CAT score more than 10 and CAT score less than 10 (iv) Total antioxidant capacity (TAC) of plasma of smokers with COPD and non-smokers with COPD (v) Total antioxidant capacity (TAC) of plasma of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification (vi) Total antioxidant capacity (TAC) of plasma of COPD patients classified as mild, moderate, severe and very severe based on GOLD score (vii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing cough and COPD patients not experiencing cough (viii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness (ix) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

5.3.3 Comparative analysis of COPD patients based on age: COPD patients with age less than the median age (median age = 58) and COPD patients with age more than the median age

Table 5.21 Statistical analysis using unpaired t-test of COPD patients with age less than median age and with age more than median age.

Table Analyzed	Col: Unpaired t test - <MEDIAN AGE VS >MEDIAN AGE
Column B	> than median age
vs.	vs.
Column A	< than median age
Unpaired t test	
P value	0.4328
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.7876, df=98
How big is the difference?	
Mean of column A	62.50
Mean of column B	51.08

Difference between means (B - A) ± SEM	-11.43 ± 14.51
95% confidence interval	-40.22 to 17.36
R squared (eta squared)	0.006290
F test to compare variances	
F, DFn, Dfd	2.114, 51, 47
P value	0.0105
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	52
Sample size, column B	48

In summary, table 5.21 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients with age less than median age and with age more than median age. The mean concentration (equivalent to ascorbic acid) for COPD patients with age less than median age and with age more than median age were 62.50 ± 0.32 µg/ml and 51.08 ± 0.33 µg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients with age less than median age and with age more than median age did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of µg/ml of ascorbic acid equivalent.

5.3.4 Comparative analysis of COPD patients based on CAT score: COPD patients having a CAT score of less than 10 and COPD patients having a CAT score of more than 10

Table 5.22 Statistical analysis using unpaired t-test of COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10	
Table Analyzed	Col: Unpaired t test
Column B	CAT score > 10

vs.	vs.
Column A	CAT score < 10
Unpaired t test	
P value	0.1220
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.560, df=98
How big is the difference?	
Mean of column A	111.9
Mean of column B	54.73
Difference between means (B - A) ± SEM	-57.18 ± 36.65
95% confidence interval	-129.9 to 15.56
R squared (eta squared)	0.02423
F test to compare variances	
F, DFn, Dfd	2.121, 3, 95
P value	0.2053
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	96

In summary, table 5.22 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10. The mean concentration (equivalent to ascorbic acid) for COPD patients having CAT score less than 10 and COPD patients having CAT score more

than 10 were 111.9 ± 0.44 µg/ml and 54.73 ± 0.32 µg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10 did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of µg/ml of ascorbic acid equivalent.

5.3.5 Comparative Analysis of Smoker and Non-smoker COPD Patients

Table 5.23 Statistical analysis using unpaired t-test of non-smokers with COPD and smokers with COPD

Table Analyzed	Col: Unpaired t test - non-smokers vs smokers
Column B	Smokers
vs.	vs.
Column A	Non-smokers
Unpaired t test	
P value	0.3934
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8573, df=98
How big is the difference?	
Mean of column A	62.09
Mean of column B	49.41
Difference between means (B - A) ± SEM	-12.68 ± 14.79
95% confidence interval	-42.02 to 16.67
R squared (eta squared)	0.007443
F test to compare variances	
F, DFn, Dfd	4.032, 59, 39

P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	60
Sample size, column B	40

In summary, table 5.23 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from non-smoker COPD patients and smokers with COPD. The mean concentration (equivalent to ascorbic acid) for non-smoker COPD patients and smokers with COPD were 62.09 ± 0.32 μg/ml and 49.41 ± 0.33 μg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3.6 Comparative analysis of COPD patients based on ABE classification: COPD patients classified as group A, B and E

Table 5.24 Statistical analysis using one-way ANOVA of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification

Table Analyzed	Col: One-way ANOVA - ABE classification				
Data sets analyzed	A-C				
ANOVA summary					
F	2.837				
P value	0.0635				
P value summary	ns				

Significant diff. among means (P < 0.05)?	No				
R squared	0.05527				
Brown-Forsythe test					
F (DFn, DFd)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	28636	2	14318	F (2, 97) = 2.837	P=0.0635
Residual (within columns)	489488	97	5046		
Total	518124	99			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	100				

In summary, table 5.24 shows analysis of one-way ANOVA performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients classified as GOLD group A, GOLD group B and

GOLD group E based on ABE classification. The mean concentration (equivalent to ascorbic acid) of COPD patients classified as GOLD group A, GOLD group B and GOLD group E were 107.17 ± 0.40 μg/ml, 38.04 ± 0.32 μg/ml and 44.86 ± 0.58 μg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients classified as GOLD group A, GOLD group B and GOLD group E did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3.7 Comparative analysis of COPD patients based on GOLD score: COPD patients classified as mild, moderate, severe and very severe

Table 5.25 Statistical analysis using one-way ANOVA of COPD patients classified as mild, moderate, severe and very severe based on GOLD score

Table Analyzed	Col: One-way ANOVA - GOLD score				
Data sets analyzed	A-D				
ANOVA summary					
F	1.219				
P value	0.3072				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.03668				
Brown-Forsythe test					
F (DFn, DFd)	2.063 (3, 96)				
P value	0.1103				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				

Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	19007	3	6336	F (3, 96) = 1.219	P=0.3072
Residual (within columns)	499117	96	5199		
Total	518124	99			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	100				

Summing up, table 5.25 shows analysis of one-way ANOVA performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients classified as mild, moderate, severe and very severe based on GOLD score. The mean concentration (equivalent to ascorbic acid) for COPD patients classified as mild, moderate, severe and very severe were 58.50 ± 0.47 μg/ml, 41.60 ± 0.33 μg/ml, 38.67 ± 0.31 μg/ml and 51.16 ± 0.32 μg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3.8 Comparative analysis of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness

Table 5.26 Statistical analysis using unpaired t-test of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness	
Table Analyzed	Col: Unpaired t test
Column B	Breathlessness
vs.	vs.
Column A	Non-breathlessness
Unpaired t test	
P value	0.8473
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.1930, df=98
How big is the difference?	
Mean of column A	52.26
Mean of column B	57.43
Difference between means (B - A) ± SEM	5.173 ± 26.80
95% confidence interval	-48.00 to 58.35
R squared (eta squared)	0.0003801
F test to compare variances	
F, DFn, Dfd	5.375, 91, 7
P value	0.0247
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	8
Sample size, column B	92

Summing up, table 5.26 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness were 57.43 ± 0.33 μg/ml and 52.26 ± 0.25 μg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

5.3.9 Comparative analysis of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

Table 5.27 Statistical analysis using one-way ANOVA of COPD patients experiencing mucous and COPD patients not experiencing mucous

Table Analyzed	Col: Unpaired t test - No mucous vs mucous
Column B	Mucous
vs.	vs.
Column A	No mucous
Unpaired t test	
P value	0.7624
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.3032, df=98
How big is the difference?	
Mean of column A	62.07
Mean of column B	56.06
Difference between means (B - A) ± SEM	-6.011 ± 19.82

95% confidence interval	-45.35 to 33.33
R squared (eta squared)	0.0009373
F test to compare variances	
F, DFn, Dfd	1.560, 15, 83
P value	0.2071
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	16
Sample size, column B	84

Summing up, table 5.27 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing mucous production and COPD patients not experiencing mucous were 56.06 ± 0.31 µg/ml and 62.07 ± 0.38 µg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of µg/ml of ascorbic acid equivalent.

5.3.10 Comparative analysis of COPD patients experiencing cough and COPD patients not experiencing cough

Table 5.28 Statistical analysis using unpaired t-test of COPD patients experiencing cough and COPD patients not experiencing cough	
Table Analyzed	Col: Unpaired t test - No cough vs cough
Column B	Cough
vs.	vs.

Column A	No cough
Unpaired t test	
P value	0.3595
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9207, df=98
How big is the difference?	
Mean of column A	74.26
Mean of column B	54.44
Difference between means (B - A) ± SEM	-19.82 ± 21.53
95% confidence interval	-62.54 to 22.90
R squared (eta squared)	0.008575
F test to compare variances	
F, DFn, Dfd	1.837, 12, 86
P value	0.1089
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	13
Sample size, column B	87

Summing up, table 5.28 shows analysis of unpaired t-test performed on the data obtained from FRAP assay which was used to measure the TAC (Total Antioxidant Capacity) of human blood plasma obtained from COPD patients experiencing cough and COPD patients not experiencing cough. The mean concentration (equivalent to ascorbic acid) for COPD patients experiencing cough and COPD patients not experiencing cough were 54.44 ± 0.31 µg/ml and 74.26 ± 0.33 µg/ml respectively using FRAP assay. The antioxidant capacity of plasma obtained from

COPD patients experiencing cough and COPD patients not experiencing cough did not show significant difference ($p < 0.05$). The plasma TAC was expressed in terms of µg/ml of ascorbic acid equivalent.

5.4 Griess Assay

Concentration of nitrite in COPD patients and healthy controls were measured using the Griess assay. Unpaired t-test and one-way ANOVA was performed using GraphPad Prism 9 software on data obtained using Griess assay. LSD test was performed using Costat software to compare the means of concentration of nitrite in COPD patients and healthy controls.

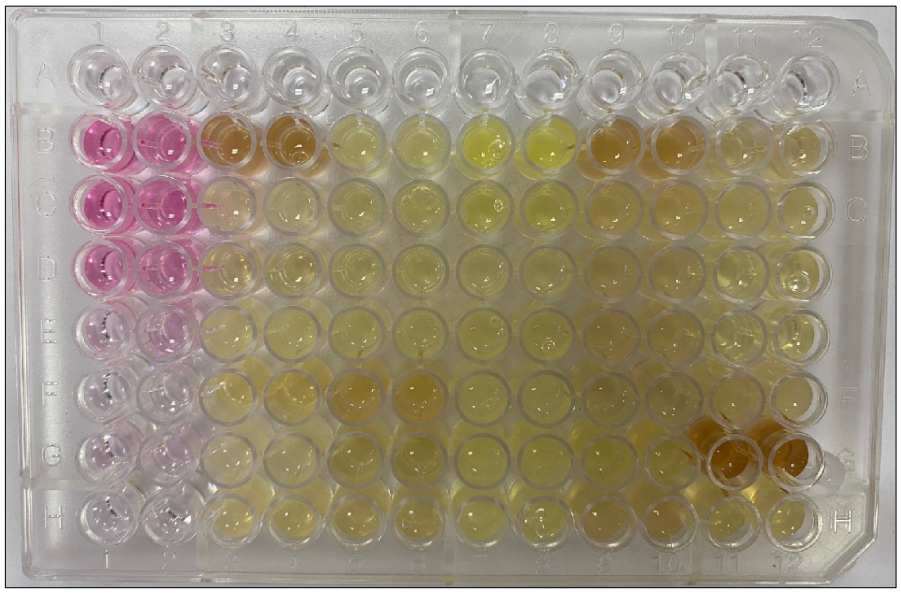


Figure 5.11 ELISA plate used for performing Griess Assay

In summary, figure 5.11 shows the ELISA plate used for performing Griess Assay in which wells A1 to A12 contained blank, wells B1-B2 to H1-H2 contained different concentration of nitrite for plotting standard curve and rest of the wells B3 to H4, B5 to H6, B7 to H8, B9 to H10 and B11 to H12 contained samples.

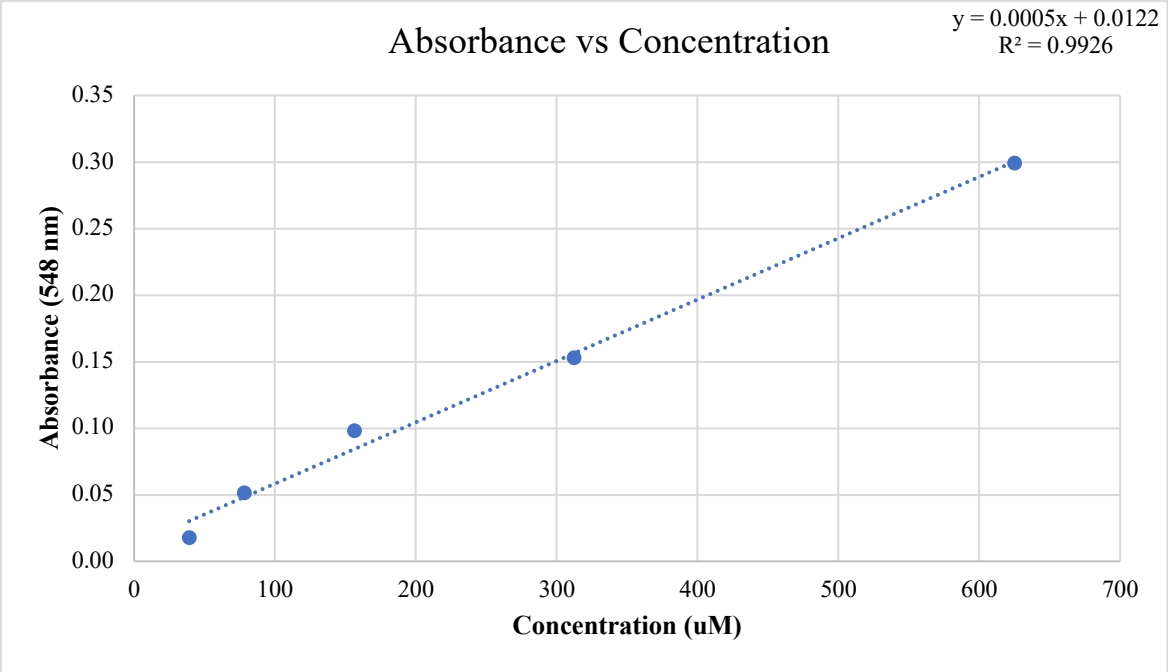


Figure 5.12 Standard curve of Sodium Nitrite for Griess Assay

The calibration curve is needed to calculate samples concentrations. The concentration of protein (µM) was determined using the equation y = 0.0005x + 0.0122 with an R^2 value of 0.9926, where y is the absorbance and x is concentration as shown in the graph (Fig 5.12)

Table 5.29 Nitrite concentrations for Standard curve	
Nitrite Concentration (µM)	**Absorbance**
0	0
39.0625	0.02
78.125	0.05
156.25	0.10
312.5	0.15
625	0.30

5.4.1 Comparative Analysis of COPD Patients and healthy controls

The concentrations (equivalent to nitrite) of patients and healthy controls obtained through the Griess assay were analyzed using Co stat software. Compare means test was applied to the data of patients and healthy controls separately.

'Multiple Comparison of Means' tests compare several means and organize the means into subgroups of not-significantly-different means. The significance level is the level of uncertainty (usually 0.05 or 0.01) that the means declared to be different are indeed different. LSD test was performed to compare the means. The test requires a reasonable estimate of the variance (usually from an ANOVA) of the population being tested. The test requires columns of data with the means, the names of the means, and the sample sizes.

Table 5.30 Concentration (along with SD) of patients arranged in descending order

S. No.	*Patient code no.*	*Concentration*
1	6	2266.93 a ± 0.82
2	37	2264.93 a ± 0.82
3	104	2193.27 ab ± 0.47
4	1	2100.6 abc ± 0.47
5	8	2093.93 abc ± 0.47
6	28	2058.93 abc ± 0.47
7	7	2010.93 abcd ± 0.47
8	64	1827.93 abcd ± 0.82
9	98	1824.93 abcd ± 0.94
10	2	1788.6 abcd ± 0.47
11	20	1773.27 abcd ± 0.82
12	29	1727.6 abcd ± 0.82
13	88	1712.93 abcd ± 0.82
14	92	1707.6 abcd ± 0.94
15	77	1656.6 abcd ± 0.82
16	100	1614.93 abcd ± 0.94
17	23	1577.6 abcd ± 0.82
18	81	1564.6 abcd ± 0.82
19	90	1563.6 abcd ± 0.94
20	72	1549.27 abcd ± 0.47
21	30	1548.6 abcd ± 0.94
22	50	1541.6 abcd ± 0.47
23	97	1531.27 abcd ± 0.47

24	107	1526.93 abcd ± 0.94
25	87	1512.27 abcd ± 0.82
26	59	1505.6 abcd ± 0.47
27	67	1483.27 abcd ± 0.82
28	19	1464.27 abcd ± 0.82
29	141	1397.93 abcd ± 0.47
30	94	1383.27 abcd ± 0.82
31	118	1350.27 abcd ± 0.82
32	43	1318.93 abcd ± 0.82
33	25	1262.93 abcd ± 0.82
34	31	1240.27 abcd ± 0.82
35	38	1214.93 abcd ± 0.47
36	95	1205.27 abcd ± 0.47
37	76	1203.27 abcd ± 0.00
38	56	1197.93 abcd ± 0.47
39	26	1195.6 abcd ± 0.82
40	117	1179.6 abcd ± 0.82
41	63	1161.6 abcd ± 0.94
42	124	1151.93 abcd ± 0.00
43	116	1150.93 abcd ± 0.47
44	40	1140.93 abcd ± 0.00
45	129	1137.93 abcd ± 0.47
46	70	1114.27 abcd ± 0.82
47	69	1106.6 abcd ± 0.82
48	61	1082.6 abcd ± 0.47
49	39	1073.93 abcd ± 0.82
50	33	1072.27 abcd ± 0.00
51	154	1036.27 abcd ± 0.47
52	9	1032.27 abcd ± 0.47
53	93	1004.27 abcd ± 0.94
54	134	958.93 abcd ± 0.47
55	136	916.93 abcd ± 0.82

56	5	903.27 abcd ± 0.82
57	86	882.93 abcd ± 0.47
58	140	853.93 abcd ± 0.47
59	113	850.27 abcd ± 0.47
60	131	845.93 abcd ± 0.82
61	150	834.6 abcd ± 0.47
62	71	822.6 abcd ± 0.47
63	108	807.27 abcd ± 0.94
64	65	780.27 abcd ± 0.94
65	80	779.27 abcd ± 0.47
66	110	775.27 abcd ± 0.94
67	156	767.6 abcd ± 0.47
68	42	765.6 abcd ± 0.94
69	96	724.6 abcd ± 0.94
70	62	724.6 abcd ± 0.47
71	127	672.6 abcd ± 0.47
72	68	672.6 abcd ± 0.94
73	78	671.6 abcd ± 0.00
74	99	654.93 abcd ± 0.94
75	85	635.93 abcd ± 0.94
76	84	598.6 abcd ± 0.94
77	48	596.6 abcd ± 0.47
78	143	588.6 abcd ± 0.94
79	101	582.93 abcd ± 0.47
80	147	574.6 abcd ± 0.82
81	146	563.6 abcd ± 0.82
82	148	548.6 abcd ± 0.47
83	119	514.27 abcd ± 0.47
84	57	513.6 abcd ± 0.00
85	22	503.6 abcd ± 0.94
86	138	502.93 abcd ± 0.94
87	122	484.93 abcd ± 0.47

88	123	468.27 abcd ± 0.94
89	149	465.6 abcd ± 0.47
90	21	461.6 abcd ± 0.47
91	75	440.6 abcd ± 0.82
92	144	430.6 bcd ± 0.00
93	125	423.93 bcd ± 0.00
94	14	401.6 bcd ± 0.00
95	137	393.27 bcd ± 0.00
96	126	366.6 bcd ± 0.82
97	103	356.93 cd ± 0.82
98	66	331.6 cd ± 0.47
99	132	209.6 d ± 0.00
100	155	185.6 d ± 0.82

In summary, the concentration of patients obtained using the Griess assay ranged from 2266.93 to 185.6. The highest concentration was shown by patient number 6 viz., 2266.93 a ± 0.82. The lowest concentration was shown by patients number 155 viz., 185.6 d ± 0.82.

Table 5.31 Concentration (along with SD) of controls arranged in descending order

S. No.	*Control code no.*	*Concentration*
1	15	3203.9 a ± 0.82
2	17	2955.6 ab ± 0.94
3	16	2675.9 abc ± 0.47
4	34	2582.9 abc ± 1.25
5	13	2484.9 abc ± 0.47
6	32	2343.9 abc ± 0.00
7	109	2238.6 abc ± 0.00
8	52	2206.6 abc ± 0.47
9	73	2104.6 abc ± 0.47
10	186	2081.6 abc ± 0.47
11	139	1984.3 abc ± 1.25

12	177	1971.6 abc ± 0.94
13	3	1886.6 abc ± 0.82
14	31	1878.9 abc ± 0.82
15	24	1654.9 abc ± 0.00
16	18	1643.6 abc ± 0.94
17	152	1639.3 abc ± 0.94
18	55	1615.6 abc ± 0.82
19	51	1564.3 abc ± 0.47
20	82	1560.6 abc ± 0.00
21	45	1534.9 abc ± 0.82
22	79	1491.9 abc ± 0.94
23	4	1429.9 abc ± 0.82
24	36	1429.6 abc ± 0.94
25	41	1373.6 abc ± 0.82
26	53	1285.3 abc ± 0.94
27	105	1266.9 abc ± 0.94
28	188	1265.9 abc ± 0.82
29	106	1253.3 abc ± 0.94
30	121	1158.6 abc ± 0.00
31	175	1145.6 abc ± 1.25
32	83	1112.9 abc ± 0.47
33	191	1097.6 abc ± 0.82
34	35	1052.6 abc ± 0.47
35	27	1033.3 abc ± 0.82
36	185	1026.6 abc ± 0.47
37	145	1021.6 abc ± 0.82
38	120	1015.3 abc ± 0.47
39	114	980.3 abc ± 0.47
40	189	968.9 abc ± 0.82
41	182	934.3 abc ± 0.94
42	190	862.6 abc ± 0.82
43	184	847.6 abc ± 0.82

44	176	784.6 abc ± 0.82
45	183	746.6 abc ± 0.00
46	153	710.9 bc ± 0.47
47	135	628.6 bc ± 0.82
48	179	598.3 bc ± 0.47
49	192	588.3 bc ± 0.94
50	174	568.3 bc ± 0.82
51	130	557.3 bc ± 0.82
52	111	516.9 bc ± 0.00
53	196	512.6 bc ± 0.82
54	178	480.6 c ± 0.00
55	112	479.6 c ± 0.47
56	187	465.6 c ± 1.25
57	169	464.6 c ± 0.82
58	102	407.6 c ± 0.82
59	181	366.9 c ± 0.94
60	180	346.6 c ± 0.00

In summary, the concentration of nitrite in healthy controls obtained using the Griess assay ranged from 3203.9 to 346.6. The highest concentration was shown by control number 15 viz., 3203.9 ± 0.82. The lowest concentration was shown by control number 180 viz., 346.6 ± 0.00.

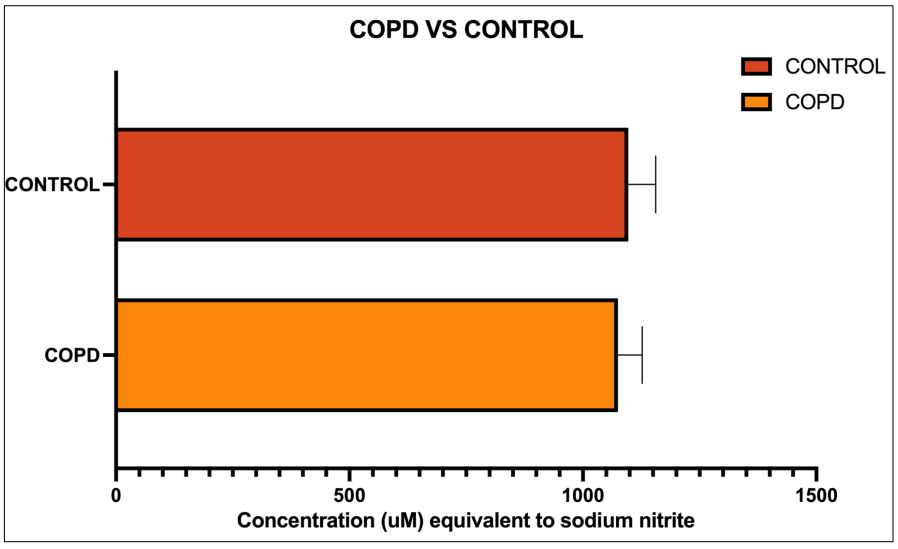


Figure 5.13 Concentration of Nitrite of plasma of healthy controls and COPD patients

In summary, the above figure 5.13 shows the mean of concentration of nitrite in the plasma obtained from the COPD patients corresponded to 1072.22 ± 0.62 µg/ml of nitrite equivalent. The mean concentration of nitrite in plasma obtained from healthy control corresponded to 1301.52 ± 0.67 µg/ml of nitrite equivalent. The concentration of nitrite in plasma obtained from healthy volunteers and patients with COPD showed significant difference ($p < 0.05$).

Table 5.32 Statistical analysis using unpaired t-test of 100 COPD patients and 60 healthy control.

Table Analyzed	Col: Unpaired t test - COPD VS CON
Column B	CONTROL
vs.	vs.
Column A	COPD
Unpaired t test	
P value	0.0193
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.365, df=158
How big is the difference?	
Mean of column A	1072
Mean of column B	1302
Difference between means (B - A) ± SEM	229.3 ± 96.97
95% confidence interval	37.77 to 420.8
R squared (eta squared)	0.03418
F test to compare variances	
F, DFn, Dfd	1.811, 59, 99
P value	0.0090
P value summary	**

Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	100
Sample size, column B	60

In summary, table 5.32 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma of COPD patients and healthy controls. The mean concentration of nitrite in COPD patients and healthy controls were 1072.12 ± 0.04 μM and 1302 ± 0.02 μM respectively using Griess assay. The mean concentration of nitrite in plasma obtained from healthy volunteers and patients with COPD did showed significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.2 Comparative Analysis of Male and Female COPD Patients

Table 5.33 Statistical analysis using unpaired t-test of female and male COPD patients	
Table Analyzed	Col: Unpaired t test - Male vs Female
Column B	Female
vs.	vs.
Column A	Male
Unpaired t test	
P value	0.6499
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.4553, df=98
How big is the difference?	

Mean of column A	1086
Mean of column B	1032
Difference between means (B - A) ± SEM	-54.22 ± 119.1
95% confidence interval	-290.5 to 182.1
R squared (eta squared)	0.002110
F test to compare variances	
F, DFn, Dfd	1.331, 25, 73
P value	0.3469
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	74
Sample size, column B	26

In summary, table 5.33 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from the female COPD patients and male patients with COPD. The mean concentration of nitrite for female COPD patients and male patients with COPD were 1032 ± 0.32 μM and 1086 ± 0.04 μM respectively using Griess assay. The concentration of nitrite in plasma obtained from female COPD patients and male patients with COPD did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

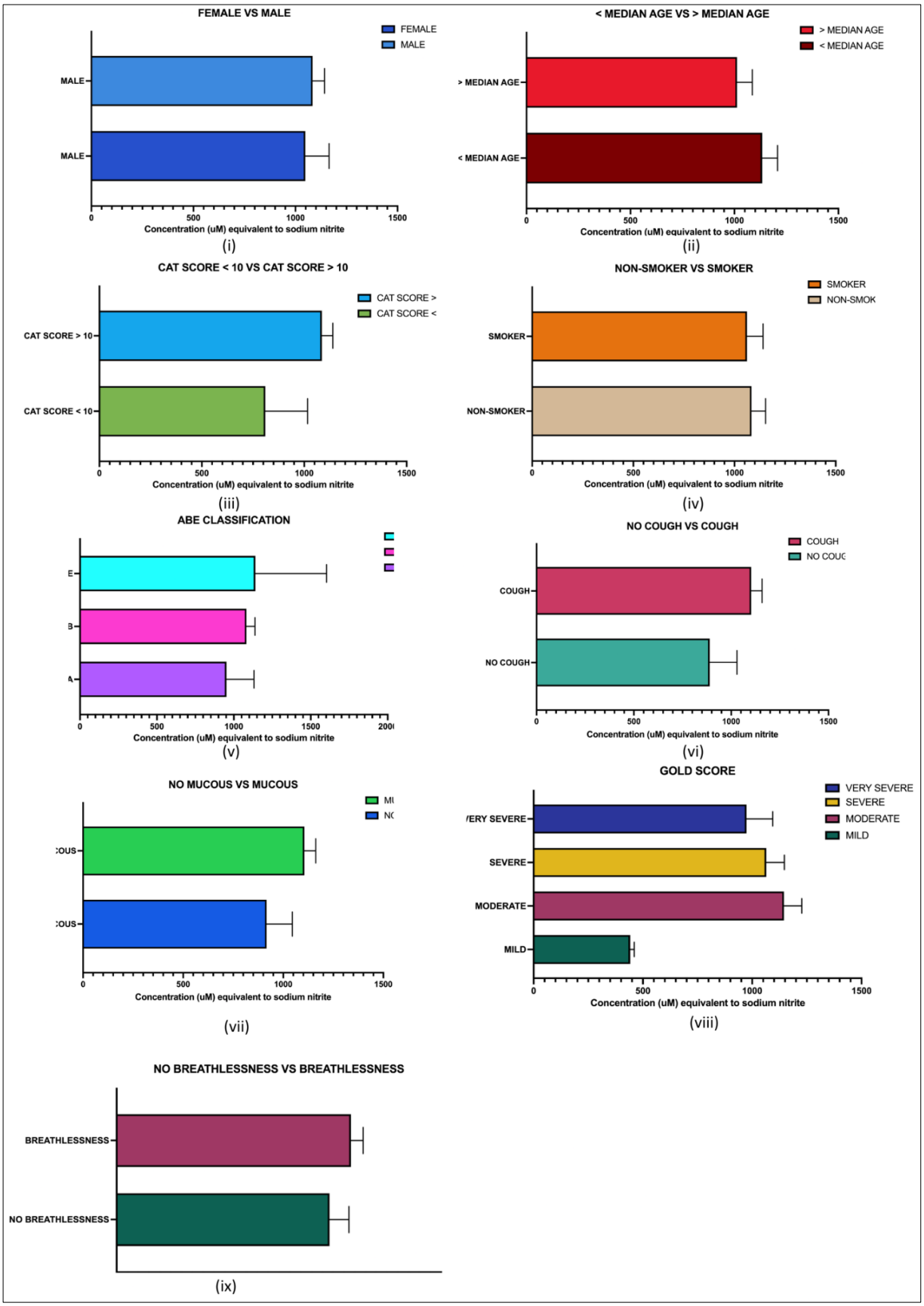


Figure 5.14 Graph showing comparative analysis of COPD patients on the basis of various parameters (i) Total antioxidant capacity (TAC) of plasma of female and male COPD patients (ii) Total antioxidant capacity (TAC) of plasma of COPD patients with age less than median age and with age more than median

age (iii) Total antioxidant capacity (TAC) of plasma of COPD patients with CAT score more than 10 and CAT score less than 10 (iv) Total antioxidant capacity (TAC) of plasma of smokers with COPD and non-smokers with COPD (v) Total antioxidant capacity (TAC) of plasma of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification (vi) Total antioxidant capacity (TAC) of plasma of COPD patients classified as mild, moderate, severe and very severe based on GOLD score (vii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing cough and COPD patients not experiencing cough (viii) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness (ix) Total antioxidant capacity (TAC) of plasma of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

5.4.3 Comparative analysis of COPD patients based on age: COPD patients with age less than the median age (median age = 58) and COPD patients with age more than the median age

Table 5.34 Statistical analysis using unpaired t-test of COPD patients with age less than median age and with age more than median age.

Table Analyzed	Col: Unpaired t test – less than median age vs more than median age
Column B	less than median age
vs.	vs.
Column A	more than median age
Unpaired t test	
P value	0.2579
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.138, df=98
How big is the difference?	
Mean of column A	1129
Mean of column B	1011

Difference between means (B - A) ± SEM	-118.3 ± 104.0
95% confidence interval	-324.7 to 88.02
R squared (eta squared)	0.01304
F test to compare variances	
F, DFn, Dfd	1.077, 51, 47
P value	0.7999
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	52
Sample size, column B	48

Summing up, table 5.34 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients with age less than median age and with age more than median age. The mean concentration of nitrite in COPD patients with age less than median age and with age more than median age were 1129 ± 0.32 μM and 1011 ± 0.33 μM respectively using Griess assay. The mean concentration of nitrite in plasma obtained from COPD patients with age less than median age and with age more than median age did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.4 Comparative analysis of COPD patients based on CAT score: COPD patients having a CAT score of less than 10 and COPD patients having a CAT score of more than 10

Table 5.35 Statistical analysis using unpaired t-test of COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10	
Table Analyzed	Col: Unpaired t test - CAT score < 10 vs CAT score > 10

Column B	CAT score > 10
vs.	vs.
Column A	CAT score < 10
Unpaired t test	
P value	0.3074
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.026, df=98
How big is the difference?	
Mean of column A	810.8
Mean of column B	1083
Difference between means (B - A) ± SEM	272.3 ± 265.4
95% confidence interval	-254.4 to 799.1
R squared (eta squared)	0.01063
F test to compare variances	
F, DFn, Dfd	1.606, 95, 3
P value	0.7960
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	96

Summing up, table 5.35 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10. The mean concentration of nitrite in COPD patients having CAT score less

than 10 and COPD patients having CAT score more than 10 were 810.8 ± 0.44 μM and 1083 ± 0.32 μM respectively using Griess assay. The mean concentration of nitrite in plasma obtained from COPD patients having CAT score less than 10 and COPD patients having CAT score more than 10 did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.5 Comparative Analysis of Smoker and Non-smoker COPD Patients

Table 5.36 Statistical analysis using unpaired t-test of non-smokers with COPD and smokers with COPD

Table Analyzed	Col: Unpaired t test - Non-smokers vs Smokers
Column B	Smokers
vs.	vs.
Column A	Non-smokers
Unpaired t test	
P value	0.9491
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.06395, df=98
How big is the difference?	
Mean of column A	1075
Mean of column B	1068
Difference between means (B - A) ± SEM	-6.826 ± 106.7
95% confidence interval	-218.6 to 205.0
R squared (eta squared)	4.173e-005
F test to compare variances	

F, DFn, Dfd	1.125, 59, 39
P value	0.7044
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	60
Sample size, column B	40

Summing up, table 5.36 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from non-smoker COPD patients and smokers with COPD. The mean concentration of nitrite in non-smoker COPD patients and smokers with COPD were 1075 ± 0.32 μM and 1068 ± 0.33 μM respectively using Griess assay. The mean concentration of nitrite in plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.6 Comparative analysis of COPD patients based on ABE classification: COPD patients classified as group A, B and E

Table 5.37 Statistical analysis using one-way ANOVA of COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification

Table Analyzed	Col: One-way ANOVA - ABE classification				
Data sets analyzed	A-C				
ANOVA summary					
F	0.1057				

P value	0.8998				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.002175				
Brown-Forsythe test					
F (DFn, DFd)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	58285	2	29142	F (2, 97) = 0.1057	P=0.8998
Residual (within columns)	26738152	97	275651		
Total	26796437	99			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	100				

Summing up, table 5.37 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients classified as GOLD group A, GOLD group B and GOLD group E based on ABE classification. The mean concentration of nitrite in COPD patients classified as GOLD group A, GOLD group B and GOLD group E were 979.99 ± 0.40 μM, 1077.43 ± 0.32 μM and 1140.93 ± 0.58 μM respectively using Griess assay. The antioxidant capacity of plasma obtained from COPD patients classified as GOLD group A, GOLD group B and GOLD group E did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.7 Comparative analysis of COPD patients based on GOLD score: COPD patients classified as mild, moderate, severe and very severe

Table 5.38 Statistical analysis using one-way ANOVA of COPD patients classified as mild, moderate, severe and very severe based on GOLD score

Table Analyzed	Col: One-way ANOVA - GOLD score				
Data sets analyzed	A-D				
ANOVA summary					
F	1.486				
P value	0.2233				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.04437				
Brown-Forsythe test					
F (DFn, DFd)	1.574 (3, 96)				
P value	0.2007				

P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1189075	3	396358	F (3, 96) = 1.486	P=0.2233
Residual (within columns)	25607362	96	266743		
Total	26796437	99			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	100				

Summing up, table 5.38 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients classified as mild, moderate, severe and very severe based on GOLD score. The mean concentration of nitrite in COPD patients classified as mild, moderate, severe and very severe were 442.77 ± 0.47 μM, 1146.03 ± 0.33 μM, 1056.34 ± 0.31 μM and 974.95 ± 0.32 μM respectively using Griess assay. The antioxidant capacity of plasma obtained from non-smoker COPD patients and smokers with COPD did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.8 Comparative analysis of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness

Table 5.39 Statistical analysis using unpaired t-test of COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness	
Table Analyzed	Col: Unpaired t test - Breathlessness vs No breathlessness
Column B	No breathlessness
vs.	vs.
Column A	Breathlessness
Unpaired t test	
P value	0.4772
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.7134, df=105
How big is the difference?	
Mean of column A	1080
Mean of column B	975.0
Difference between means (B - A) ± SEM	-104.9 ± 147.1
95% confidence interval	-396.6 to 186.7
R squared (eta squared)	0.004823
F test to compare variances	
F, DFn, Dfd	1.340, 91, 14
P value	0.5547
P value summary	ns

Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	92
Sample size, column B	15

Summing up, table 5.39 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness. The mean concentration of nitrite in COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness were 1080 ± 0.33 μM and 975.0 ± 0.25 μM respectively using Griess assay. The antioxidant capacity of plasma obtained from COPD patients experiencing breathlessness and COPD patients not experiencing breathlessness did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.9 Comparative analysis of COPD patients experiencing mucous production and COPD patients not experiencing mucous production

Table 5.40 Statistical analysis using one-way ANOVA of COPD patients experiencing mucous and COPD patients not experiencing mucous

Table Analyzed	Col: Unpaired t test - Mucous vs No mucous
Column B	No mucous
vs.	vs.
Column A	Mucous
Unpaired t test	
P value	0.1895
P value summary	ns
Significantly different (P < 0.05)?	No

One- or two-tailed P value?	Two-tailed
t, df	t=1.321, df=98
How big is the difference?	
Mean of column A	1102
Mean of column B	915.3
Difference between means (B - A) ± SEM	-186.8 ± 141.4
95% confidence interval	-467.4 to 93.77
R squared (eta squared)	0.01750
F test to compare variances	
F, DFn, Dfd	1.031, 83, 15
P value	
P value summary	
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	84
Sample size, column B	16

Summing up, table 5.40 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous. The mean concentration of nitrite in COPD patients experiencing mucous production and COPD patients not experiencing mucous were 1102 ± 0.31 μM and 915.3 ± 0.38 μM respectively using Griess assay. The antioxidant capacity of plasma obtained from COPD patients experiencing mucous production and COPD patients not experiencing mucous did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of μM.

5.4.10 Comparative analysis of COPD patients experiencing cough and COPD patients not experiencing cough

Table 5.41 Statistical analysis using unpaired t-test of COPD patients experiencing cough and COPD patients not experiencing cough	
Table Analyzed	Col: Unpaired t test - Cough vs No cough
Column B	No cough
vs.	vs.
Column A	Cough
Unpaired t test	
P value	0.1720
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.376, df=98
How big is the difference?	
Mean of column A	1100
Mean of column B	887.9
Difference between means (B - A) ± SEM	-211.9 ± 154.0
95% confidence interval	-517.5 to 93.71
R squared (eta squared)	0.01895
F test to compare variances	
F, DFn, Dfd	1.082, 86, 12
P value	0.9455
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	87
Sample size, column B	13

Summing up, table 5.41 shows analysis of unpaired t-test performed on the data obtained from Griess assay which was used to measure the concentration of nitrite in human blood plasma obtained from COPD patients experiencing cough and COPD patients not experiencing cough. The mean concentration of nitrite in COPD patients experiencing cough and COPD patients not experiencing cough were 1100 ± 0.31 µM and 887.9 ± 0.33 µM respectively using FRAP assay. The antioxidant capacity of plasma obtained from COPD patients experiencing cough and COPD patients not experiencing cough did not show significant difference ($p < 0.05$). The concentration of nitrite in plasma was expressed in terms of µM.

5.5 Catalase In-gel Activity Assay

Qualitative analysis of catalase in COPD patients and healthy controls was performed by analysing the running native gel. The results obtained by native gel were quantified using ImageJ software.

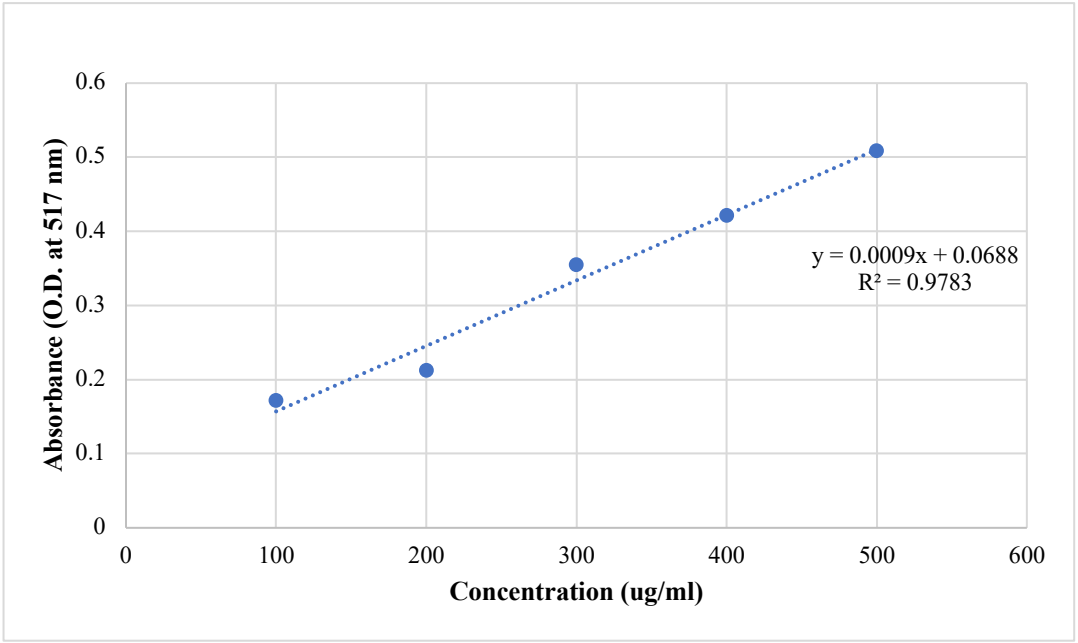


Figure 5.15 Standard curve of BSA (Bovine Serum Albumin) for protein estimation

The calibration curve is needed to calculate samples concentrations. The concentration of protein (µg/ml) was determined using the equation $y = 0.0009x + 0.0688$ with an R^2 value of 0.9783, where y is the absorbance and x is concentration as shown in the graph (Fig 5.15)

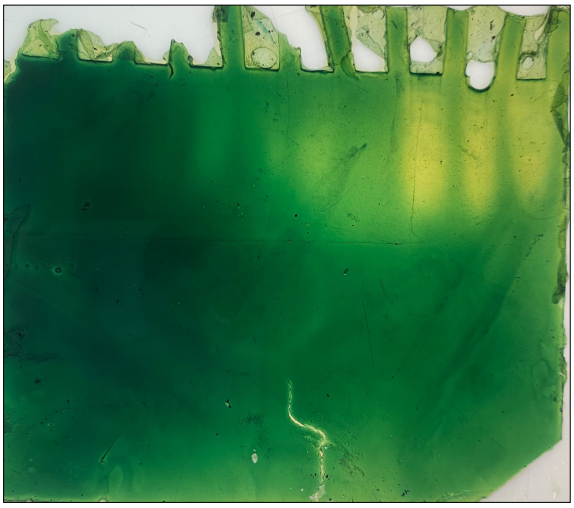


Figure 5.16 Results of native gel run; from right to left well 1 contained control number 170, well 2 contained control number 170 due to spillage, well 3 contained COPD case number 107, well 5 contained COPD case number 108, well 7 contained COPD case number 110, and well 9 contained COPD case number 113

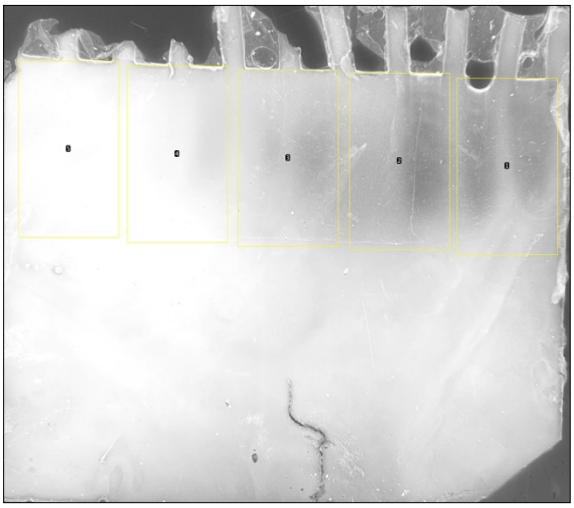


Figure 5.17 Results of native gel analyzed using ImageJ software; from right to left well 1 contained control number 170, well 2 contained control number 170 due to spillage, well 3 contained COPD case number 107, well 5 contained COPD case number 108, well 7 contained COPD case number 110, and well 9 contained COPD case number 113

The results after running native gel were analyzed using ImageJ. ImageJ is a public domain software. It is used to process and analyze scientific photographs. Different peaks depending on the intensity of the smeared bands were obtained by analyzing the area selected under the

well. Area under the graph was calculated using ImageJ software. This helped quantify the amount of catalase present in the samples run in different wells. Area covered was directly proportional to the amount of catalase present in the samples. The more the area covered, the more the amount of catalase.

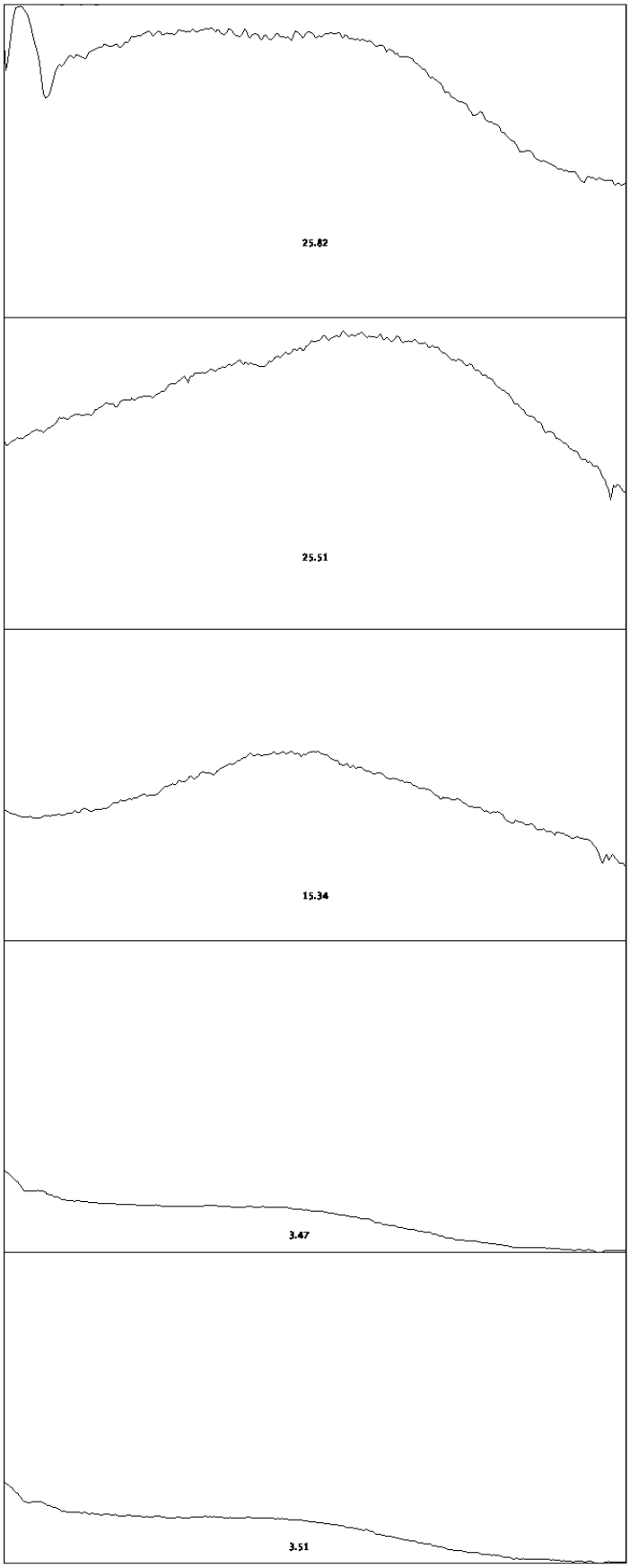


Figure 5.18 Peaks obtained by analyzing the area selected on the native gel using ImageJ software; area under the peaks were calculated using ImageJ software

Table 5.42 Statistical analysis using unpaired t-test of COPD patients and healthy controls	
Table Analyzed	Col: Unpaired t test
Column B	Control
vs.	vs.
Column A	COPD
Unpaired t test	
P value	0.0201
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.392, df=57
How big is the difference?	
Mean of column A	31.76
Mean of column B	56.81
Difference between means (B - A) ± SEM	25.05 ± 10.47
95% confidence interval	4.079 to 46.03
R squared (eta squared)	0.09121
F test to compare variances	
F, DFn, Dfd	1.839, 7, 50
P value	0.2004
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	51
Sample size, column B	8

Summing up, table 5.42 shows analysis of unpaired t-test performed on the area under the curve obtained from ImageJ software after the analysis of result of native gel carried out using Catalase In-gel activity assay of human blood plasma of COPD patients and healthy controls. The mean concentration equivalent to ascorbic acid for COPD patients and healthy controls were 31.76 ± 0.04 μg/ml and 56.81 ± 0.02 μg/ml respectively using DPPH assay. The antioxidant capacity of plasma obtained from healthy volunteers and patients with COPD showed significant difference ($p < 0.05$). The plasma TAC was expressed in terms of μg/ml of ascorbic acid equivalent.

CHAPTER 6

DISCUSSION

6. Discussion

The aim of this dissertation was to evaluate various biomarkers related to oxidative stress and antioxidant capacity in patients with Chronic Obstructive Pulmonary Disease (COPD) compared to healthy controls. The biomarkers assessed in this study were Total Antioxidant Capacity (TAC) using DPPH and FRAP Assay, Nitrite levels using Griess Assay, and Catalase activity using Catalase In-Gel Activity Assay. The study focused on exploring potential biomarkers that could indicate COPD sensitivity. COPD is a chronic inflammatory lung disease characterized by oxidative stress, which can lead to tissue damage and disease progression. Assessing biomarkers related to oxidative stress and antioxidant capacity can provide valuable insights into COPD pathophysiology and its association with disease severity.

The DPPH assay was employed to determine the Total Antioxidant Capacity (TAC) in plasma samples from COPD patients and healthy controls. The assay measures the ability of antioxidants in the samples to scavenge the stable free radical DPPH. The TAC was expressed as µg/ml equivalent to ascorbic acid. Ascorbic acid, a well-known antioxidant, was used to establish a standard curve for comparison. The DPPH assay is a widely used method to evaluate the overall antioxidant capacity of a sample. The results of this assay can provide valuable insights into the balance between oxidants and antioxidants in COPD patients compared to healthy individuals. Any significant differences observed in TAC levels between the two groups could indicate alterations in the oxidative stress status in COPD.

The results of the DPPH assay showed that the mean TAC in COPD patients was 10.12 ± 0.04 µg/ml, while in healthy controls, it was 11.03 ± 0.02 µg/ml. However, the difference in TAC between COPD patients and healthy controls was not statistically significant ($p > 0.05$). Similarly, no significant difference in TAC was observed when comparing male and female COPD patients, COPD patients with different age groups, and COPD patients with different CAT scores ($p > 0.05$). Furthermore, the TAC did not significantly differ between smokers and non-smokers with COPD ($p > 0.05$).

The findings of the study suggest that the TAC measured through the DPPH assay may not be a reliable biomarker to distinguish COPD patients from healthy individuals or to differentiate between different subgroups of COPD patients based on age, gender, smoking history, or CAT scores. This implies that the DPPH assay might not be sensitive enough to capture the subtle

variations in antioxidant capacity in COPD patients. Alternatively, it is possible that other factors not assessed in this study might play a more significant role in determining TAC levels in COPD patients.

For FRAP assay, a standard curve of ascorbic acid was established, and the absorbance values were measured at 593 nm. The results indicated significant differences in the TAC between COPD patients and healthy controls. The study's key results showed that the TAC of plasma in COPD patients ranged from 317.16 μg/ml to 0 μg/ml, with the highest concentration observed in patient number 78 (317.16 ± 0.21 μg/ml). In contrast, the lowest concentration was found in several patients (numbers 9, 14, 19, etc.) as 0 ± 0.00 μg/ml. On the other hand, the TAC of plasma in healthy controls ranged from 164.49 μg/ml to 0 μg/ml, with the highest concentration in control number 45 (164.49 ± 0.21 μg/ml) and the lowest in controls 13, 15, 16, etc. (0 ± 0.00 μg/ml).

The mean TAC of plasma in COPD patients was significantly higher (57.02 ± 0.27 μg/ml) compared to healthy controls (23.43 ± 0.22 μg/ml). This difference was confirmed by the statistical analysis using an unpaired t-test, which yielded a p-value of 0.0007, indicating a significant difference between the two groups. The results suggest that there is a notable alteration in the antioxidant capacity of plasma in COPD patients compared to healthy individuals. The increased TAC in COPD patients might be attributed to the oxidative stress and inflammation commonly observed in COPD, leading to an upregulation of antioxidant defences. The findings are relevant as they provide valuable insights into the antioxidant status of COPD patients, which could have implications for disease progression and management. These results are particularly relevant for clinicians and researchers working in the field of COPD, as they shed light on the potential role of antioxidant therapies in managing COPD patients. Antioxidant-based therapies might prove beneficial in reducing oxidative stress and inflammation, thereby slowing disease progression and improving overall patient outcomes.

Nitrite levels were measured using the Griess assay, which is a simple and reliable method for determining the concentration of nitrite, an important marker of nitric oxide (NO) production. Nitric oxide is involved in various physiological processes, including inflammation and vasodilation. In COPD, increased oxidative stress can lead to elevated NO production, which may contribute to disease pathogenesis. Measuring nitrite levels can provide insights into the involvement of NO in COPD and its potential role as a biomarker for COPD sensitivity.

Elevated levels of nitrite in COPD patients compared to healthy controls could suggest increased inflammation and oxidative stress in the disease.

The lower concentration of nitrites in the plasma of COPD patients suggests a potential alteration in the nitric oxide pathway in these individuals. Nitric oxide is an important signalling molecule with various physiological functions, including vasodilation and immune regulation. The decrease in nitrite levels may have implications for the overall health of COPD patients and could be related to their disease pathogenesis. The findings of lower nitrite concentrations in COPD patients may have several implications. Firstly, it could indicate impaired nitric oxide signalling in these patients, which might contribute to their respiratory symptoms and disease progression. Secondly, nitric oxide is involved in regulating blood flow and inflammation, so altered levels could impact the cardiovascular and inflammatory status of COPD patients. Understanding these implications could open new avenues for therapeutic interventions targeting the nitric oxide pathway in COPD.

Catalase activity, an important antioxidant enzyme, was assessed using the Catalase In-Gel Activity Assay. Catalase plays a crucial role in neutralizing reactive oxygen species (ROS) and hydrogen peroxide, thereby protecting cells from oxidative damage. A dysregulation in catalase activity could lead to an imbalance between ROS production and antioxidant defence. The green-blue colour and light bands in the catalase activity gel indicate the presence of catalase enzyme, which removes peroxides from the gel area, preventing the formation of a Prussian blue precipitate. The increase in bands corresponds to higher catalase activity. The analysis of the native gel using ImageJ software allowed quantifying the catalase activity in different samples. The area under the graph was directly proportional to the amount of catalase present in the samples, with a higher area indicating higher catalase levels.

The results of the Catalase In-gel Assay have significant implications. They suggest that there is a difference in catalase activity between COPD patients and healthy controls. Catalase is an important antioxidant enzyme that protects cells from oxidative damage caused by reactive oxygen species (ROS). The lower catalase activity observed in COPD patients may contribute to increased oxidative stress and lung damage, leading to the progression of the disease. Understanding the differences in catalase activity between COPD patients and healthy individuals could open up new avenues for targeted therapies. Enhancing catalase activity through medications or lifestyle modifications might potentially help mitigate oxidative stress and slow down disease progression in COPD patients. Moreover, the identified biomarkers

(TAC, nitrite, and catalase) can potentially serve as non-invasive indicators of COPD sensitivity and disease severity, aiding in early diagnosis and personalized treatment approaches. However, further studies with larger sample sizes and longitudinal designs are required to validate these findings and establish the clinical utility of these biomarkers. As with any research study, this dissertation has certain limitations that should be considered. The cross-sectional design may restrict the establishment of causality between the biomarkers and COPD. Longitudinal studies would provide more robust evidence for the role of these biomarkers in COPD progression.

Additionally, various factors such as diet, medication, and lifestyle choices might influence the antioxidant capacity and oxidative stress levels, which were not controlled for in this study. Hence, further investigations with comprehensive control of confounding variables are warranted. In conclusion, this dissertation aimed to evaluate Total Antioxidant Capacity (TAC), nitrite levels, and catalase activity as potential biomarkers for COPD sensitivity. The findings from this study could contribute to a better understanding of the role of oxidative stress and antioxidant defence in COPD pathophysiology and may have clinical implications for the management of COPD patients. Future research building upon these findings could pave the way for novel therapeutic strategies and personalized approaches for COPD patients.

CHAPTER 7

CONCLUSION

7. Conclusion

This dissertation aimed to evaluate various biomarkers related to oxidative stress and antioxidant capacity in patients with Chronic Obstructive Pulmonary Disease (COPD) compared to healthy controls. The biomarkers assessed in this study were Total Antioxidant Capacity (TAC) using DPPH and FRAP Assay, Nitrite levels using Griess Assay, and Catalase activity using Catalase In-Gel Activity Assay. The investigation into Total Antioxidant Capacity (TAC) using DPPH revealed no significant difference in TAC levels between COPD patients and healthy controls and using FRAP revealed significant difference in TAC levels between COPD patients and healthy controls. However, the Griess Assay for Nitrite levels demonstrated a significantly lower concentration of nitrites in the plasma of COPD patients compared to healthy controls. Additionally, the Catalase In-Gel Activity Assay indicated a significant difference in catalase activity between COPD patients and healthy controls, suggesting altered antioxidant defence in COPD.

The implications of these findings are multifaceted. The altered nitrite concentrations and catalase activity in COPD patients may reflect imbalances in oxidative stress and antioxidant defence mechanisms. This could potentially contribute to the pathogenesis and disease progression of COPD. The study highlights the importance of understanding oxidative stress in COPD, as it may have significant clinical implications for therapeutic interventions and disease management. However, the study has its limitations, including a relatively small sample size and a cross-sectional design. Further research with larger cohorts and longitudinal designs is warranted to validate and expand upon these findings. Moreover, the study focused on specific biomarkers, and other antioxidants and oxidative stress markers should be explored to gain a more comprehensive view of the oxidative status in COPD.

In conclusion, this dissertation contributes valuable insights into the oxidative stress and antioxidant capacity in COPD patients. The findings emphasize the complexity of oxidative stress mechanisms in COPD and the potential significance of altered nitrite concentrations and catalase activity. These results may guide future research in developing targeted therapeutic approaches for managing COPD patients and improving their overall health outcomes. Further investigations are needed to explore the interplay between oxidative stress and COPD

pathogenesis, laying the foundation for personalized treatment strategies and advancing our understanding of this chronic respiratory condition.

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Evaluation of Oxidant-Antioxidant Imbalance, Level of Nitrite, and Level of Catalase as Biomarker in Plasma Samples of Chronic Obstructive Pulmonary Disease (COPD) Patients
A Thesis submitted

CHAPTER 8

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