CHAPTER 1: INTRODUCTION

1.1 General

Chronic obstructive pulmonary disease (COPD) is a serious lung disease and it is a major threat to wellness of modern society. COPD is defined as chronic airflow obstruction that is progressive and only partly reversible (Pauwels et al., 2001). COPD includes chronic bronchitis and pulmonary emphysema. Chronic bronchitis is a chronic inflammatory condition in the lungs that causes the respiratory passages to be swollen and irritated, and there is an increase in the mucus and cough production in the respiratory passage. Increased cough and sputum production arise from an innate immune response to inhaled toxic particles and gases of cigarette smoke (Hogg, 2004).

In case of emphysema destruction of the airway walls results in the enlargement of the distal airspaces due to that there is less surface area for gas exchange and the person feels breathless (Snider et al., 1985). The beginnings of clinical understanding of the chronic bronchitis i.e. a part of the COPD can be traced to Badham (1814), he used the word catarrh which refers to the chronic cough and hypersecretion of mucus. Laennec (1821) described the emphysema component of COPD in his book entitled “Treatise of diseases of the chest”. In emphysema cases, Laennec (1821) found that lungs were hyperinflated and did not empty well.

Global Initiative on Chronic Obstructive Lung Disease (GOLD) established latest definition of COPD and recently adopted by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) i.e. COPD is a “preventable and treatable disease state characterised by airflow limitation that is not fully reversible’’ (Pauwels et al., 2001; Celli and MacNee, 2004).

1.1.1 Global Scenario of COPD

It is estimated that in 2020, out of 68 million deaths worldwide, 11.9 million will be caused by lung diseases: 4.7 by COPD, 2.5 by pneumonia, 2.4 by...
TB and 2.3 million by lung cancer (GBD, 2015). At current time, COPD is the fourth most common cause of death in the United States of America (NHLBI Data Fact Sheet, 2003). The world is estimated to be inhabited by a record of 7.3 billion people with COPD in 2015 (Lopez-Campos et al., 2016). The GBD survey highlighted that COPD was the sixth leading cause of death in 1990 and the fourth since 2000 (Lopez-Campos et al., 2016). By 2020 it is expected that COPD will become the third most common cause of death (NHLBI Data Fact Sheet, 2003).

Lung diseases cause loss in the health-care budgets within the EU with direct costs of approximately €47.3 billion. Around $32.1 billion loss in USA economy in direct and indirect costs in 2003 as a result of COPD (NHLBI Data Fact Sheet, 2003). The four major respiratory diseases including COPD, asthma, pneumonia and TB have medical costs totalling €38.7, €17.7, €10.1 and €2.1 billion, respectively (European Respiratory Society, 2014).

According to the Korean Health Insurance Review and Assessment Service database, similar situation of COPD cases found in Asia. Medical costs per person were US$ 2803 ± 3865 in 2009 in Korea (Kim et al., 2014). This study also revealed that patient’s number with high-grade COPD increased very rapidly in Korea and they showed a high prevalence of co-morbid disease. The total medical costs increased three times higher in patients with high-grade COPD as compared to those without it (Lopez-Campos et al., 2016).

1.1.2 COPD burden in India

Indian researchers have studied COPD prevalence over the last 5 decades. These surveys were started from local level and could not be generalized on a national level (Bhome, 2012). According to WHO, India contributes a growing percentage of COPD cases which is estimated to be amongst the highest in the world; i.e. more than 64.7 estimated age standardized death rate per 100,000 amongst both sexes (WHO, 2012). This study showed approximately 556,000 cases in India (>20%) out of a world total of 2,748,000 every year (Lopez et al., 2006). Salvi and Aggarwal (2012) proposed that COPD kills half a million people
in India every year, more than those who die due to tuberculosis, malaria or HIV-AIDS.

The National Commission on Macroeconomics and Health studied various diseases and their burden on the health care system of the India in 2001. COPD burden was very shocking and eye-opener for policy makers and clinicians. This commission started estimates of COPD population from 1996 and projected upto 2016 as per census of India statistics. The number of COPD cases in the country is to rise from 17.0 million in the year 2006 to 22.2 million by 2016 (Report of the National Commission on Macroeconomics and Health, 2005). Approximately 24 million adults over the age of 40 years had COPD in 2010 in India. It is expected that this number will increase 34% to approximately 32 million by 2020 (Planning Commission of Government of India, 2011).

The prevalence of COPD among men in India ranges between 2.12% to 9.4% in north India and 1.4% to 4.08% in South India (Jindal et al., 2001). A large multi-site study carried out by the ICMR has reported a higher prevalence of COPD among men (5.0%) than women (3.2%) aged above thirty five years (Jindal et al., 2006). A higher prevalence was observed in low income groups as compared to the well-off (5.4% vs. 3.3%) and in rural areas as compared to urban regions (4.4% vs. 3.7%) (Planning Commission of Government of India, 2011).

1.2 Pathogenesis and pathophysiology of COPD

COPD is a disorder characterized by an abnormal inflammatory response in the lungs and obstruction of expiratory airflow (Pauwels et al., 2001). This airflow limitation is slowly progressive over years. Airflow limitation is caused by several anatomical lesions, including loss of lung recoil elasticity, fibrosis and narrowing of small airways (Niewoehner and Sobonya, 1994; Spurzem and Rennard, 2005). Various other factors such as oedema of the airways, accumulation of secretions and smooth muscle contraction can also cause limitation in airflow that may be partially reversible. There are various processes involved in the pathogenesis of COPD i.e. inflammation, an imbalance between
proteases and antiproteases and an imbalance between oxidants and antioxidants in the lungs (MacNee, 2006).

1.2.1 Inflammation

COPD is characterized by a specific pattern of inflammation. Many inflammatory mediators are increased in COPD, including neutrophils, macrophages and T-lymphocytes (specifically CD8+) in different parts of the lungs, which relate to the airflow limitation (Gozum et al., 1992). During exacerbations there might be an increase in the production of eosinophils in some COPD patients (Martinez et al., 2000). The structural cells of the lung, i.e. epithelial and mesenchymal cells, are the producers of inflammatory mediators (Piquette et al., 2000). These inflammatory cells are capable of releasing various cytokines and inflammatory mediators such as leukotriene 4, interleukin 8 and tumour necrosis factor-α (MacNee, 2006).

1.2.2 Protease and antiprotease imbalance

Cigarette smoke and other possible COPD risk factors as well as inflammation can produce oxidative stress in the lung that stimulates several inflammatory cells (macrophages, neutrophils) to release a combination of proteinases and, on the other hand inactivate various antiproteinases by the process of oxidation (American Thoracic Society/European Thoracic Society, 2004). Protease and antiprotease imbalance causes the destruction of elastin which is a major connective tissue component in lung parenchyma which can lead to the emphysema (GOLD, 2006). The main proteases involved are produced by neutrophils: serine proteases elastase, cathepsin G, and protease produced by macrophages (cysteine proteases and cathepsins E, A, L, and S) and several matrix metalloproteases (MMP-8, MMP-9, and MMP-12 (MacNee, 2006). The primary antiproteinases involved in the pathogenesis of emphysema are α1 antitrypsin, secretory leucoprotease inhibitor and tissue inhibitors of metalloproteases (MacNee, 2006).
1.2.3 Oxidative stress

Imbalance between oxidants and antioxidants leads to the oxidative stress. The main source of oxidants in COPD includes cigarette smoke, and reactive oxygen and nitrogen species released from inflammatory cells. The cellular sources of reactive oxygen species in the lungs are neutrophils, eosinophils, alveolar epithelial cells, alveolar macrophages, bronchial and endothelial cells (Hakhamaneshi et al., 2007). In COPD patients oxidative stress contributes by oxidising a variety of biological molecules that can lead to cell death or dysfunctioning, damaging the biological extracellular matrix, inactivating key antioxidant defence system (or activating proteinases) or enhancing gene expression (either by activating transcription factors e.g. nuclear factor-κB) or by promoting histone acetylation (Lanken et al., 1991).

1.3 Risk factors

1.3.1 Tobacco smoke

The most common tobacco-related risk factor for COPD is cigarette smoking (GOLD, 2006). Tobacco smoke exposure is also the most significant factor for COPD with 80–90% of all cases attributable to smoking (US Surgeon General, 1984). Passive exposure to cigarette smoke may also contribute in the development of COPD by increasing the lung total burden of inhaled gases and particles (Eisner et al., 2005). Pipe and cigar smoking have associated with increased COPD risk, but less threatening than cigarette smoking (Dewar and Curry, 2006).

1.3.2 Indoor air pollution

In high and middle income countries, tobacco smoking is the primary risk factor but in lower income countries exposure of indoor air pollution, such as the use of biomass fuels for cooking and heating purpose is the main burden of COPD (WHO, 2013).
1.3.3 Occupational dusts (organic and inorganic)

Peoples having the following occupations have the higher risk of developing COPD and associated lung diseases due to the dust particles or chemicals at their jobs:

Automobile-drivers, vehicular mechanics, rubber products, metal etching, plastics, ammonia exposure of refrigeration and petroleum refining, grain dust and funguses in farmers, textile mill manufacturing, leather manufacturing, welders in automotive industries (Brashier and Kodgule, 2012).

1.3.4 Exposure to crystalline silica

Occupational exposure to crystalline silica dust occurs in many industrial places worldwide. In workplace silica particles are inhaled to lungs, these particles can initiate toxic and inflammatory processes in conducting and peripheral airways (Hnizdo and Vallyathan, 2003).

1.3.5 Outdoor air pollution

In urban areas, outdoor air pollution is a major public health problem largely due to emissions of air pollutants from both motor vehicles and industrial plants. Gauderman et al. (2004, 2007) revealed that outdoor pollution and traffic-related air pollution have harmful effect on lung development in 10–18 years aged children. Particulate pollutants such as ozone (O$_3$) and NO$_2$ can produce deleterious effects on the airway, such as increase in bronchial reactivity (Foster et al., 2000), increased airways oxidative stress (Oh et al., 2011) and pulmonary and systemic inflammation (Happo et al., 2010; Budinger et al., 2011).

1.3.6 Previous Tuberculosis

Previous history of tuberculosis is increasingly recognized as a risk factor for COPD. Some studies have revealed that tuberculosis patients, even after adequate anti-Koch’s therapy are 2-6 times more liable to develop airway obstruction (Mannino and Buist, 2007; Viegi et al., 2007; Salvi and Barnes, 2009).
1.3.7 Old age

In case of aging, the structure, function and control of the respiratory system are adversely affected. The elastic recoil which is the major determinant of maximal expiratory flow of the lungs is diminished with aging which causes increased compliance in lung at high lung volumes (Knudson, 1991; De Bisschop et al., 2005; Pride, 2005).

1.3.8 Genetic factors

Presently, only well-known genetic risk factor for COPD is SERPINA1 gene which codes for serine protease inhibitor, alfa-1 antitrypsin (AAT). Any disorder in SERPINA1 gene leads to the deficiency of AAT-1, causing uninhibited action of proteases and play an important role in the development of emphysema (Stoller and Aboussouan, 2005).

1.4 Symptoms of COPD

COPD is characterized by the inflammation of the lungs, increased mucous production and distruction of the small airway walls. The common symptoms of COPD are:

1.4.1 Chronic cough

Chronic cough commonly is the first symptom in the development of COPD. Cough is often ignored by the patient as an expected consequence of smoking or environmental exposures. Initially, the cough may be occasionally, but later on it is present throughout the day.

1.4.2 Wheezing and chest tightness

When the person exhale through narrow or obstructed air passages, he often hear a whistling or musical sound that is called wheezing. Wheezing and chest tightness are nonspecific symptoms that may vary between days. These symptoms may be present at the mild stage of COPD.
1.4.3 Shortness of breath (Dyspnea)

Dyspnea is the major symptom of COPD. During increased physical activity the shortness of breath oftenly occurs in the patients with COPD, due to that daily task, such as walking, household chores, dressing, or bathing becomes more difficult.

1.4.4 Fatigue

In COPD patients due to difficulty in breathing the patients cannot get enough oxygen to their blood and muscles. Without necessary oxygen person body slow down and fatigue sets in. Patients may feel tired because of the extra efforts of lungs for getting oxygen in and the carbon dioxide out.

The symptoms may vary from person to person, the symptom get worse according to the severity of disease i.e. from moderate COPD to severe COPD (GOLD, 2006).

1.5 Diagnosis of COPD

Several pulmonary function tests are used to measure the breathing ability of the subjects for the diagnosis of COPD.

1.5.1 Arterial blood gas testing: Arterial blood gas test is used to evaluate the measurements of oxygen level, carbon dioxide (effectiveness of respiration), and several other parameters. This test is used to evaluate the effectiveness of breathing (National Jewish Health, 2015).

1.5.2 Bronchial provocation test: This test is used to measure the sensitivity of the airways in lungs. A Spirometry breathing test is performed before and after the inhalation of a spray such as methacholine. spirometry results are used to compare before and after inhalation of the spray to see the changes in breathing ability (National Jewish Health, 2015).

1.5.3 Spirometry test: The quickest and easiest method for diagnosis of COPD is the spirometry test. In this test subject breath into a large hose connected to a machine, called a spirometer. The spirometer measures the amount of much air
that lungs can hold and how fast lungs can blow out air. This indicates the airway obstruction whether present or not. Spirometry test results are useful in making the diagnosis of a specific lung disorder. Spirometry test should measure the volume of air exhaled forcibly from the point of maximal inhaled air (Forced vital capacity, FVC), the volume of air exhaled during the first second (forced expiratory volume in one second, FEV1) and the ratio of these two measurements (FEV1/FVC) should be calculated. Spirometric measurements are evaluated by comparison with reference values based on age, height, sex and race (GOLD, 2006).

1.6 Stages of COPD

GOLD (2006) classifications are used to describe the severity of the obstruction or airflow limitation.

**Stage I: Mild COPD** - Characterized by mild airflow limitation (FEV1/FVC < 70%, FEV1 ≥ 80% predicted). In this stage symptoms of chronic cough and sputum production may be present. At this stage, the person is usually unaware that his or her lung function is abnormal.

**Stage II: Moderate COPD** – Moderate COPD patients have worsening airflow limitation (FEV1/FVC < 70%, 50% ≤ FEV1 < 80% predicted,), in this stage patient may become breathless when walking on level surfaces and have to stop to catch breath. This is the stage at which patients typically seek medical attention because of the chronic respiratory symptoms or an exacerbation of their disease.

**Stage III: Severe COPD** - In this stage further worsening of airflow limitation occurs (FEV1/FVC < 70%; 30% < FEV1 < 50% predicted), there is greater shortness of breath, patients have less exercise capacity and repeated exacerbations, fatigue is increased.

**Stage IV: Very severe COPD** – In this type of COPD severe airflow limitation occurs (FEV1/FVC < 70%; FEV1 < 30% predicted or FEV1 < 50% predicted)
and the person becomes breathless during everyday activity. Breathing trouble may even be life-threatening in some cases.

There is considerable evidence for increased oxidative stress among COPD patients which can lead to the DNA damage (Repine et al., 1997; MacNee, 2001; Neofytou et al., 2012; Gandhi and Kaur, 2015). However, a few studies were reported that have examined the DNA damage among COPD patients with the help of comet assay (Ceylan et al., 2006; Maluf et al., 2007; da Silva et al., 2013) and for the evaluation of total antioxidant activity of plasma with the help of FRAP assay (Hakhamaneshi et al., 2007; Emin et al., 2010) and assessment of genetic damage with the help of MN assay (Casella et al., 2006; Maluf et al., 2007; da Silva et al., 2013). In India the work related to DNA damage in COPD patients employing MN assay and comet assay is lacking while there are several studies that have recently come up in regard to FRAP assay on COPD patients (Nadeem et al., 2005; Ahmad et al., 2013; Waseem et al., 2015). However in Haryana work on COPD patients with regard to nuclear anomalies and DNA damage is altogether lacking.

Keeping in view the above the present study was undertaken to analyse the genetic damage in buccal epithelial cells and the peripheral blood lymphocytes (PBLs) and to evaluate total antioxidant activity of plasma in COPD patients with the following objectives:

1.7 Objectives:
1. To conduct an epidemiological survey of the COPD human subjects and healthy matched controls.
2. To compare the frequency of micronuclei in buccal epithelial cells of the COPD patients with controls.
3. To detect genetic damage, if any, using comet assay in the COPD human subjects along with healthy matched controls.
4. To estimate the total antioxidant activity in the COPD human subjects along with healthy matched controls.
5. To provide a baseline data based on which further studies may be planned.