REVIEW OF LITERATURE

In the present case-control study comprising Chronic Obstructive Pulmonary Disease (COPD) cases and healthy controls, a multitude of end-points assessing genetic damage, oxidative stress, lipemic profile, alpha-1-antitrypsin activity and genetic polymorphisms have been studied. The approach for conducting the study comprised firstly the identification of COPD cases from among those with occupational exposure from activities performed during stone-crushing. Subsequently, three different tissues (venous blood, buccal mucosa and urine) were sampled and processed for assessment of genetic damage as COPD cases often progress to malignancy. Blood sera samples were also analyzed for levels of oxidative stress, alpha-1-antitrypsin activity and dyslipidemia. Molecular genotyping from genomic whole blood DNA was performed for some variants of a COPD candidate gene SERPINA1 (Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 1) and the metabolic (Glutathione-S-transferases, GSTs) genes which may act via their gene products as predispositional factors for disease-causation and/or genetic damage. To rule-out bias for risk identification from occupational setting, similar assessment was conducted on an age-, gender- and socio-economic status -matched healthy group of similar ethnic origin.

Accordingly, first an overview on COPD with emphasis on occupational causation-factors and on Genetics of COPD is presented followed by review of literature on studies on genetic damage, oxidative stress and dyslipidemia in COPD cases. Finally is presented an up-to-date review on association studies in COPD cases on selected molecular genotypes in the context of the present study and studies on the genotoxicity of soil constituents.

2.1 Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease, first described in 1964 (Mitchell and Filley, 1964), is a complex, multifactorial, polygenic disease, characterized by progressive irreversible air-flow obstruction caused by the combination of chronic bronchitis and emphysema (WHO, 2016). Chronic bronchitis is the presence of chronic productive cough for three months each in two successive years, and emphysema is characterized
by the permanent enlargement of air-spaces distal to the terminal bronchioles with the destruction of the bronchiole walls (American Thoracic Society, 1995). The disease is manifested on destruction of the alveolar compartments whereby air-flow limitation occurs resulting in reduced oxygen exchange in the lungs (Boue et al., 2015). The measurement of air-flow in and out of the lungs, characterizes the disease and is the basis of the pulmonary function test (http://www.copdgene.org/information-copd accessed on April 7, 2016).

2.1.1 COPD Presentation and Diagnosis

**Presentation** - COPD cases present with cough, sputum production, dyspnea and impaired exercise tolerance. There is a chronic and abnormal inflammatory response of the lungs (Angelis et al., 2014) and the disease reflects from a complex interplay between genetic and environmental interactions (El-Zein et al., 2012).

**Diagnosis** - The spirometric evidence (pulmonary function test) of air-flow limitation confirms the diagnosis of COPD (Celli and MacNee., 2004; Miller and Levy, 2015) although the symptoms of cough, or sputum-production, or a history of exposure to risk factors also have to be considered for identification of the disease (Vestbo et al., 2006).

**The Pulmonary Function Test** - A spirometer is used to test the lung-function capacity, and by considering the forced expiratory volume in one second (FEV₁) and the forced vital capacity (FVC), obstructive lung disease can be diagnosed as per the recommendations of the Global Initiative for Chronic Obstructive Pulmonary Disease these assist in categorizing COPD severity depending upon the FEV₁/FVC ratio and the FEV₁ measurements. COPD is defined by the A<0.7 ratio of FEV₁/FVC defines COPD. For categorization of COPD by severity (GOLD, 2010; 2016), the per cent FEV₁ predicted value is considered i.e.

- mild COPD cases having ≥80% of predicted FEV₁
- moderate cases with 50-80% of predicted FEV₁
- severe cases with 50-30% of predicted FEV₁
- very severe cases with ≤ 30% of predicted FEV₁

2.2 COPD Prevalence
Chronic Obstructive Pulmonary Disease is one of the most common chronic respiratory diseases and is the leading cause of death, presently ranked fourth after heart diseases, paralysis and diabetes but in all probably should rise to the third by 2030 because of its increasing prevalence (GOLD, 2014; Prakash et al., 2015).

**Global Scenario**- Accounting to the Burden of Obstructive Lung Diseases (BOLD) study, carried out in 12 countries there is 10% prevalence of COPD (Buist et al., 2007). However wide variation (Buist et al., 2008; Prakash et al., 2015) with 23-20% prevalence (30.30% in men, 19.00% in women) in South Africa (Jithoo et al., 2006), 19.10% (28.00% in men,10.30% in women) in Turkey (Kocabas et al., 2006) with 16.20% in Uppasala, Sweden (Danielsson et al., 2012), 14.20% in Lisbon, Portugal (Bábaraa et al., 2013) and 10.00% (11.9% in men and 8.80% in women) in Tehran, Iran (Sharifi et al., 2014). In the U.S.A. alone, there are >13 million physician-diagnosed COPD patients (Ford et al., 2013; NHLBI, 2014).

On studying the global prevalence of air flow-obstruction, there is also considerable variation from 4.20% in Nampicuan (The Philippines) to 23.00% in men in Cape Town, South Africa through 5.70% in Pune (India) and 20.70% in women in Upp Sala, Sweden (Burney et al., 2014).

Regarding stage I COPD, in Salzburg, Austria, its prevalence was 6.10% and of stage II, 10.70% (Schirnhofer et al., 2007) with 7.80% and 4.02%, respectively in Tunisia (Daldoul et al., 2013).

**Indian Scenario**- Nation-wide prevalence data for COPD on the basis of spirometry are not available probably because of the size and diversity of the Indian population (Gupta et al., 2013). Using a validated questionnaire-based method (Gupta et al., 2014), COPD prevalence rates varied for gender (3.20% in females, 5.00% in males) in a multicentric study from Bangalore, Chandigarh, Delhi and Kanpur (Jindal, 2006; McKay et al., 2012). The 5.10% prevalence in rural areas near Pune comprised 6.50% male and 3.40% female cases (Salvi et al., 2007) and higher prevalence (19.30%) was reported from Kashmir (Koul, 2013) though spirometrically-diagnosed COPD cases in another study (17% in males and 15% in females) were less (Burney et al., 2014).

Earlier reports have documented COPD-prevalence ranging from 2.12%-9.40% in males and 1.33%-4.90% in females from North India (Jindal et al., 2004) with
somewhat differences from South India (1.40% - 4.08% in males and 2.55-2.70% in females) as per WHO-Government of India Biennium (WHO Factsheet, 2004). According to the Indian Study on Epidemiology of Asthma, Respiratory Symptoms and Chronic Bronchitis in Adults (INSEARCH), COPD prevalence is 3.70% (4.50% male and 2.90% female cases), with a total estimated burden of COPD of ~15 million (Gupta et al., 2014). Singh, (2014) has there are ~30% COPD cases with and that 500,000 deaths annually are from COPD. In Punjab it has been observed that more COPD patients visit hospitals for treatments during burning of the rice-stubble (Bharti, 2015).

2.3 Missing-COPD” Cases

As many as 50-80% of COPD cases are missed-out because of misdiagnosis or co-current diagnosis and rely only on reported symptoms which are not sufficiently sensitive and / or because of failure of persons to report to the health provider (Levy et al., 2009). Therefore, in situ workplace COPD identification gains significance and also because estimates reveal that work-related burden of COPD accounts for ~15-20% of all the COPD cases (Barnes et al., 2003; Omland et al., 2014).

2.3.1 Under-estimated and Undiagnosed COPD Cases

The disease is under-estimated and under-diagnosed worldwide mostly because of gaps in disease- recognition and management (O’Donnell et al., 2003; Camp et al., 2008). In India, the attributable main reason is disparity in availability and affordability of health care facilities (Gupta et al., 2013; 2014). In Spain, the rate of under-diagnosis is 78% (Pena et al., 2000) with 89% in Sweden (Lindberg et al., 2005) and 88% in Austria (Schirnhofer et al., 2007).

2.4 Pre-disposition to COPD

The disease has hereditary and environmental components with heritability estimates varying from 40-75% (El-Zein et al., 2012). The COPD gene map to-date mentions 192 genes and 16 loci (Bossé, 2012; Probert et al., 2015) and on the basis of Genome Wide Association Studies (GWAS), 125 genes have shown association with COPD (Probert et al., 2015). Among important environmental triggers of COPD are exposure to cigarette smoke (Laniado-Laborin, 2009; Muro, 2011), biomass cooking (Liu et al.,
2007; Perez-Padilla et al., 2014) and to dust and chemical reagents (Blanc, 2012; Fishwick et al., 2010; 2013). Other causal factors include exposure from air-pollutants at industrialized urban areas (Wichmann et al., 2007), low socio-economic status (REVIHAPP, 2013), low Body Mass Index (Cao et al., 2012) and the male gender (Han et al., 2007). However, occupational pollutants are responsible for 31% COPD cases (Hnizdo et al., 2002); these are detailed hereunder:

2.5 Workplace Exposure and COPD

The very first evidence linking COPD and occupational exposures was to dust and/or to dust and fumes (Becklake, 1985; 1989) although its etiology from smoking continued to take precedence (Buist et al., 2008) despite the fact that 25–45% of COPD cases are in non-smokers (Salvi and Barnes 2009; Omland et al., 2014). Population-based studies have attributed COPD to workplace exposures to dusts, noxious gases/vapours, and fumes (Boschetto et al., 2006) with exposure from silica dust, fibrogenic and non-fibrogenic dust and irritant vapours accounting for 95% COPD cases at the workplaces compared to 65% cases from smoking (Mazitova et al., 2012). Rather, several occupations pose risk for developing COPD (Rushton et al., 2007) and literature perusal has documented this from farming (Eduard et al., 2009; Szczyrek et al., 2011), construction (Bergdahl et al., 2004; Dement et al., 2013), exposure at foundry units (Deschamps, 2013), in coal miners (Santo, 2011) and tunnel workers (Ulvestad et al., 2000). Details about independent factors triggering COPD are described below:

**Smoking** - Cigarette smoking is the most common tobacco-related risk factor for COPD (Laniado-Laborín, 2009; Mohammad and El Maghraby, 2014), which with passive exposure also contributes to the development of COPD by increasing the lung total burden of inhaled particles and gases (Eisner et al., 2005).

**Indoor pollution/Biomass Fuel** - As many as 50% of all households and 90% of rural households rely on biomass fuel (wood, charcoal, other vegetable matter, and animal dung) and coal as their main sources of domestic energy. More than 80% of homes in China, India, and sub-Saharan Africa use biomass fuel for cooking and 30-75% in rural areas of Latin America (Salvi and Barnes, 2009). The fuels release air-pollutants like sulphur dioxide (SO₂), carbon monoxide (CO), nitric oxide (NO₂), formaldehyde and
particulate matter (PM) in the ambient indoor air, which can lead to manifestation of COPD (Salvi et al., 2012).

**Outdoor Pollution** - Air-pollution in most cities of Asia, South America, and Africa has increased as a result of industry and traffic-congestion (Salvi and Barnes, 2009). The increased concentrations of both, gaseous and PM in the urban areas have exhibited association with increased respiratory morbidity and cardiovascular mortality, and possibly COPD (Sunyer, 2001).

**Occupational Exposure** - There exists a strong relation between COPD incidence and exposure to various hazardous emittants in the workplace environment such as to silica dust, coal dust, gases and fumes (Cho et al., 2009). Exposure to inorganic dust (asbestos, cement dust, concrete dust, man-made mineral fibers, or quartz), gases and irritants (organic solvents, epoxy resins, or diisocyanates), fumes (metal fumes, asphalt fumes, or diesel exhaust), and in workplaces to wood dust such as from construction (Bergdahl et al., 2004; Dement et al., 2015), coal-mining (Santo, 2011), farming (Eduard et al., 2009; Szczyrek et al., 2011) and foundry works (Deschamps, 2013) pose risk for COPD.

**Socio-economic status** - Poor socioeconomic status (SES), which is also indicative of other factors such as intrauterine growth retardation, poor nutrition and housing conditions, is a causal factor for COPD (Hegewald and Crapo, 2007). The impact of low SES on respiratory disease in general, has been attributed to poorer housing, more hazardous occupational exposure, poorer diet, a higher prevalence of smoking, and respiratory infections during childhood (Kartaloglu, 2013).

**Low-birth weight babies and Infections** - Babies born to mothers exposed to environmental tobacco smoke have low birth weight (Boy et al., 2002) Low birth weight as an independent risk factor for COPD, is associated with poor lung growth and lung function during childhood and adulthood (Hancox et al., 2009). Indoor burning of wood and animal dung and other biofuels cause infections (in lower respiratory tract of the low- birth weight babies who survive), and is an important cause of death of children in developing countries, with the heaviest burden (42%) of such deaths being in Asia (Salvi and Barnes, 2009).

### 2.6 Genetics of COPD
The overall phenotype in COPD is from epistatic (additive or synergistic) interactions (Wood and Stockley, 2006) though the first reported and only gene having association with COPD was the alpha-1-antitrypsin (Eriksson, 1964), renamed as Serpin peptidase inhibitor, clade A, member 1 (SERPINA1) located on chromosome 14q32.1 (http://www.genecards.org/cgi-bin/carddisp.pl?gene= SERPINA1). The gene encodes a serine protease inhibitor that protects against proteolytic stress in the lung (Stoller and Aboussouan, 2005). Linkage analysis had revealed another gene, the SERPINE2 (2q), also as a COPD-susceptibility gene (De Meo et al., 2006). Meta-analyses have however also revealed other genes with COPD association and with lung function, which are involved in the inflammatory pathways (IL4, IL6, IL13, IL1B, IL1RN, LTA, TNF, and TGFB1), protease/antiprotease activity (MMP9, TIMP2, and SERPINA3), oxidative stress (GSTM1, GSTP1, GSTT1, EPHX1, SOD2, and SOD3) and those controlling and regulating (ACE and ADRB2) hypertension (Bossé, 2012). In a meta-analysis of 1996-2008 published case-control studies (Hu et al., 2008), GSTM1 emerged as a significant genetic contributor of COPD whereas GSTT1 reported no significant association. Smolonska et al. (2009; 2010) in a meta-analysis on 20 polymorphisms in 12 genes reported a significant association of TGFB1, IL1RN, VNTR polymorphisms and of TNFA genes with COPD. In a meta-analysis on studies carried out before 2008, 27 genetic variants including GSTM1, TGFB1, TNF and SOD3 showed significant association with COPD (Castaldi et al., 2010). The Genome Wide Association Studies (GWAS) have reported three genetic loci viz. CHRNA3/ CHRNA 5/IREB2 (Young et al., 2008; Pillai et al., 2009; De Meo et al., 2009; Lambrechts et al., 2010) on chromosome 15q25. HHIP (Young et al., 2010b; Van Durme et al., 2010) on chromosome 14 (14q31), and FAM13A (Young et al., 2010a) on chromosome 4 (4q22) showed association for susceptibility to COPD. In addition to these reported loci, RIN3 on 14q32, MMP3/MMP12 on 11q22, TGFB2 on 1q41 (Cho et al., 2015) and HHIP, AGER loci on 4q31, SERPINA10 and DLC on chromosome 14q32.13 (Cho et al., 2015) conferred increased risk for COPD. Recently (de Jong et al., 2015), the first Genome Wide Interaction Study (GWIS) has documented an association of Protocadherin 9 (PCDH9), UDP-N-acetyl-a-D-galactosamine: polypeptide N-acetylgalactosaminyl transferase 13(GALNT13) and Transmembrane protein 176A (TMEM176) with lung-
function decline from occupational exposures to biological dust, mineral dust, and gases and fumes.

2.6.1 SERPINA1 as a Genetic Determinant

A well-known example of a strong genetic factor predictive of the development of COPD is α1-antitrypsin (AAT) deficiency and about 1% of affected COPD patients are α-1 antitrypsin deficient being genetically predisposed to the development of the disease (El-Zein et al., 2012). The SERPINA1 Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 1 gene is a protein coding gene and encodes the AAT–serine protease inhibitor, which has shown association with COPD, though only 1–2% of patients with COPD inherit this AAT deficiency (Smolonska et al., 2009) manifesting the disease even among never smokers. Therefore other genes involved in the inflammatory pathways, protease/antiprotease pathway, and the oxidative stress pathway may also be a predispositional factors for COPD (Van Eden and Sin, 2013; Kumar et al., 2013).

2.7 Pathophysiology of COPD

COPD is a progressive chronic inflammatory disease, primarily affecting the small airways and alveoli (Cosio et al., 2009) and resulting in impaired lung function marked by a reduced forced expiratory volume in one sec and (FEV1) and to the forced expiratory volume in one sec to forced vital capacity (FEV1 / FVC) ratio (Grose and Milroy, 2011). Local inflammatory processes in the airways and lungs, initiated from gaseous/particulate matter inhalation, can lead to chronic bronchitis, small airways disease, and emphysema (Omland et al., 2014). In fact the pathophysiological hallmark of the disease is expiratory flow limitation resulting from enlargement of the alveolar air-spaces, airway-wall fibrosis, loss of elastic recoil, smooth muscle hypertrophy, goblet-cells, hyperplasia and mucus plugging (O’Donnell, 2001; Hogg, 2004). On increasing ventilation in flow-limited cases, air trapping occurs and there is dynamic lung hyperinflation above the already increased resting volumes (O’Donnell et al., 2015). The lung hyperinflation elastic and inspiratory threshold-loading of inspiratory muscles which are already burdened with increased resistive work (Papandrinopoulou et al., 2012). This leads to constrained tidal volume expansion on doing strenuous work.
Inspiratory muscles cannot build-up pressure on lung-hyperinflation and progressively this contributes to breathlessness (O’Donnell, 2001).

The pathophysiological consequences of COPD therefore constitute hypoxia, hyperinflation, emphysema, hypoxia-induced secondary polcythemia and lung and systemic inflammation, which can induce pulmonary capillary muscularization, intimal-wall thickenings, plexiform lesions, endothelial dysfunctions and apoptosis (Brashier and Kodgule, 2012).

2.8 COPD and Genomic Instability

The literature perusal has also revealed some studies on genomic instability in COPD patients. These are reviewed here chronologically.

Casella et al. (2006) assessed cytogenetic damage in cultured whole blood lymphocytes of COPD patients (n=49) and of age-, gender- and cumulative smoking history-matched controls (n=48) using the Cytochalasin micronucleus (MN) assay and also carried out analysis of and sister-chromatid-exchange (SCE) frequency in a sub-set of 25 COPD and 23 controls. No significant differences in the frequency of micronucleated (MNd) cells and SCEs were observed between the patients and controls and also not on stratification of COPD cases and the controls, for the presence or absence of co-morbidities (none, bronchodilators, cardiovascular therapy and statins). The proliferation index (PI; SCE assay) and nuclear division index (NDI;MN test) also did not differ between the two groups implying no effects on cell cultures and cell proliferation. No significant relationship was reported on correlation analysis between the frequency of MN and the per cent FEV₁ in COPD patients. The authors suggested that any oxidative stress-induced DNA damage in patients could have been eliminated via the apoptotic-pathway, realizing into no differences between the patients and the controls with respect to genetic damage.

Maluf et al. (2007) assessed genetic damage in peripheral blood lymphocytes using the Cytokinesis-block micronucleus (CBMN) and comet assay (single cell gel electrophoresis) assays. The frequency of binucleated cells with micronuclei (p=0.000), dicentric bridges (p=0.01), buds (p=0.001), and the comet value (p=0.00) in COPD patients were significantly elevated, although there were no differences in genetic damage for gender and disease-severity. No relationship of genetic damage with
airflow-obstruction, smoking or age emerged on correlation analysis. It was hypothesized that oxidative stress in lung cells constantly induces production of free radical that can cause damage to the genetic material of both, the lungs as well as of the circulating cells.

In a study conducted by Pastukh et al. (2011), a PCR-based method was used for assessment of oxidized purine base-products in some selected 200 bp sequences present in promoters and coding regions of the vascular endothelial growth factor (VEGF) gene, Transforming growth factor beta 1 (TGF-β1), Heme oxygenase (HO-1), early growth response 1 (Egr1), and β-actin genes. Oxidative damage of the mitochondrial genome was also assessed using quantitative Southern blot analysis from lung tissues taken from severe cases of COPD and healthy controls. Oxidative damage was observed in the hypoxic response element (HRE) of the VEGF promoter and VEGF mRNA content was reduced in COPD lung tissue though mitochondrial DNA content was unaltered while mitochondrial DNA strand breaks and/or abasic sites showed an increase. Probably the generated oxygen radicals caused mtDNA mutations and could also disrupt mtDNA transcription.

A study was conducted by da Silva et al. (2013a) comprising two groups of COPD patients, one group on physical exercise (PE-COPD; n = 15) and the other not on physical exercise (COPD; n = 36), along with a control group (n=51) comprising age- and gender-matched individuals but differing from the patients for body mass index, smoking status, cigarettes smoked per year, and smoking duration. Both, DNA damage in peripheral blood (comet assay) and cytogenetic effects (buccal micronucleus cytome assay) in buccal cells were studied; plasma levels of lipid peroxidation were also estimated using the thiobarbituric acid reactive species (TBARS) method. The results of the study revealed significantly elevated basal damage index (DI) assessed by the alkaline comet assay and by the neutral comet as well as by the modified comet assay (with Fpg and Endo III enzymes) in PE-COPD cases compared to the COPD patients not exercising. The residual DNA damage (induced by methyl methanesulphonate, an alkylating agent) was significantly elevated in COPD patients in comparison to the levels in the PE-COPD and the control groups. However both the COPD groups had significantly increased number of basal cells, nuclear buds and condensed chromatin cells compared to the controls, with lipid peroxidation significantly increased in COPD
patients compared to the levels in PE-COPD and control groups. On stratification for disease-severity (mild, moderate, severe and very severe COPD cases) no significant differences in genetic damage and lipid peroxidation were observed. The FEV$_1$ and FEV$_1$/FVC ratio negatively correlated with karyorrhectic cells, suggesting that patients with more severe COPD present an increment in cytogenetic damage and early cell death by apoptosis.

In a case-control study (da Silva et al., 2013b) on 51 COPD patients and age-, gender- and BMI-matched controls (n=51) of white ethnicity, DI levels (alkaline and neutral comet assays) were significantly increased and the FEV$_1$ per cent predicted, FVC per cent predicted and FEV$_1$/FVC per cent predicted were significantly (p=0.000) decreased in the COPD patients. The residual damage after methyl methanesulphonate (MMS)-treatment was also significantly higher in the patients though there were no differences on the basis of disease-severity. Lipid peroxidation levels however showed no differences between cases and controls.

Paschalaki et al. (2013) on assessing circulating endothelial progenitor cells (EPC) dysfunction by isolation and expansion of blood out-growth endothelial cells (BOEC) from peripheral blood samples of healthy non-smokers (n=18), healthy smokers (n=11) and of COPD patients (n=20; mild=3, moderate=12, severe=5), reported significantly increased DNA double-strand breaks in BOEC in COPD patients compared to the controls.

Oit-Wiscombe et al. (2013) assessed DNA damage, Poly(ADP-ribose) polymerase (PARP) activity and PARP-1 mRNA expression using the Comet Assay IV, biotinylated-NAD incorporation assay and qRT-PCR in peripheral blood mononuclear cells of non-smoking individuals (n=7), non-obstructive smokers (n=4), COPD patients in all stages (n=19; mild=3, moderate=7, severe=5, very severe=4) and those with COPD exacerbation (n=6). Per cent DNA in tail, head length, tail length and tail migration were significantly increased (p<0.001) while there was a decrease (p<0.001) in per cent DNA in the head; mean grey level and total intensity in order from healthy control individuals to non-obstructive smokers followed by patients with increasing stage of stable COPD as well as those with COPD exacerbation. A similar trend in significant (p<0.001) increase in PARP activity was reported though a significant decrease in the
PARP-1 mRNA expression in COPD cases was reported in comparison to those with no obstruction (p = 0.040). It was suggested that the increase in DNA damage could increase from oxidative stress and inflammation which can be caused mainly by increased PARP activity rather than that caused by age, by declined FEV1% or by cigarette smoke.

In a case-control study, blood/sera samples of the COPD cases (n=32) identified spirometrically among the stone-crushing workers) and healthy controls (n=19) significantly increased 8-OHdG (DNA damage), glutathione and superoxide dismutase levels were reported (Gandhi and Kaur, 2015).

DNA damage in peripheral blood lymphocytes of patients with respiratory diseases and healthy individuals using the nanoparticle (NP) and bulk versions of the non-steroid anti-inflammatory drugs (NSAIDs) of Aspirin and Ibuprofen, was assessed using the comet and the micronucleus assays (Najafzadeh et al., 2015). After treatment with aspirin nano-suspension (ASP N) and ibuprofen nano-suspension (IBU N) compared to their bulk version (micro-suspension) in both assays, DNA damage was reported to be decreased in lymphocytes compared to levels in healthy individuals, and in asthma, and COPD and lung cancer patients. DNA damage was significantly decreased in all groups, except in the asthma group on comparing ASP N-treated to untreated lymphocytes. On the other hand, DNA damage decreased in healthy individuals and in the lung cancer patients but increased in asthma and COPD patients on comparing IBU N-treated to untreated lymphocytes. The micronuclei frequency was increased in the healthy individuals and in lung cancer patients, and decreased in asthma and COPD patients after ASP N- and IBU N- treatments.

2.9 Oxidative stress and COPD

Interactions of the mechanisms of inflammation, proteolytic/antiproteolytic imbalance, oxidative stress, apoptosis, enhanced senescence of the structural cells and of defective repair processes are involved in the pathogenesis of COPD. The investigations of oxidative stress in COPD patients are described here:

Premanand et al. (2007) assessed levels of thiobarbituric reactive substances (TBARs) and observed significantly (p<0.001) higher levels in male chronic smokers with (n=20) and without (n=20) COPD in comparison to levels in non-smokers (n=20). The levels of
GSH were however significantly (p<0.001) higher in non-smokers in comparison to levels in the smokers, with and without COPD (with no differences between the latter two). The number of pack-years of smoking also showed no significant association with TBARS and GSH levels.

In a study by Bartoli et al. (2011), the MDA levels in exhaled breath condensate were assessed in patients with asthma (n = 64), bronchiectasis (n= 19), COPD (n=73) and idiopathic pulmonary fibrosis (n=38) and in healthy non-smokers which comprised the control group (n=14). The levels of MDA were observed significantly higher in all the disease- groups (except in the idiopathic pulmonary fibrosis patients) in comparison to the control group. On comparison among the patients, MDA levels were higher in COPD patients in comparison to those with asthma and in the bronchiectasis patients. There was a significant negative association between MDA levels and per cent FEV₁ in the COPD patients.

In COPD patients (n=68) stratified into those receiving standard treatment (n=23) and those additionally taking N-acetylcysteine (NAC 600mg daily dose, n=22) and those taking it twice daily (n=23), the results revealed that the pre-treatment MDA levels were increased all the patient groups indicating oxidative stress in the patients. A significant difference was however observed in levels from baseline (0 day) to post-treatment (60 days), in both those on NAC dose once daily (p=0.001) and twice daily (p=0.023), in comparison to those on standard treatment (Patil et al., 2011).

A study from South India (Arja et al., 2013) assessed oxidative stress and antioxidant enzyme levels in male 236 COPD patients and 150 controls. Significant decrease in per cent FEV₁ predicted (p=0.000) and FEV₁/FVC (p=0.000) was observed in COPD patients compared to controls although the two groups matched for smoking pack-years. In COPD patients there was also an increase by 2.21 folds in MDA levels (p=0.000) and a decrease in levels of catalase (1.39fold, p=0.000), superoxide dismutase, SOD (1.39fold,p=0.000), glutathione peroxidase (1.19 fold, p=0.000) and glutathione (1.39fold,p=0.000). A significant increase in the mean values of MDA (p=0.005) and decrease in catalase (p=0.01) and glutathione (p=0.05) were reported from COPD stages II to IV. In the patients, smoking history showed negative relation with FEV₁ / FVC (p<0.05) and positive correlation with catalase activity (p < 0.01).
In a study (Cristóvao et al., 2013) comprising stable COPD patients (n=20;>6 months without exacerbation history, 11 males and 9 females; age 71.30±7.68y), there were significantly (p<0.001) elevated MDA levels whereas there was a significant (p<0.001) decrease in vitamin C and sulphhydryl levels in comparison to levels in healthy subjects (n=50, 41.60±12.31y, 38 males, 12 females; age 41.60±12.31y). No significant differences were however observed between non-smokers and ex-smokers in COPD patients, both for the oxidative stress (MDA) and antioxidant status (vitamin C and sulphhydryl groups).

Among 120 (40-70y) study participants (Gavali et al., 2013) comprising healthy smokers (n=30) and non-smokers (n=30), COPD patients with smoking habit (n=30) and non-smoker COPD patients (N=30). The levels of SOD decreased significantly in healthy smokers (p<0.001) and COPD patients (both non-smokers, p<0.001 and smokers, p<0.001) on comparison to healthy controls who were non-smokers. However, SOD levels did not differ between the two groups of COPD patients suggesting that smoking may not be the only cause of aetiopathology of COPD. Nonetheless, there was a significant positive correlation of SOD levels with per cent FEV1 predicted in COPD patients (smokers as well as non-smokers).

Woźniak et al. (2013) in a study on COPD patients with smoking habit (n=73, 25-72y), assessed the levels of thiobarbituric acid-reactive substances (TBARS), and conjugated dienes (CDs), glutathione peroxidase (GPx) and vitamins E and A before smoking-cessation and subsequently after the first-, second- and third-months of abstinence. The two groups comprised healthy non-smokers (n=35) and 35 smokers with COPD (n=35). The predicted FEV1 and FVC values and the FEV1/FVC ratio were similar in both groups of COPD patients but were significantly (p<0.001) lower than that in the non-smokers. Lipid peroxidation (p<0.001) and conjugated dienes (p<0.01) and SOD activity (p<0.05) were significantly higher while the glutathione peroxidase (p<0.001) levels were lower in the COPD patients in comparison to the control levels. The levels of vitamins A and E were also not significantly different between the patient and the control groups.

Maheshwari et al. (2014) assessed the association between vitamin D-binding protein gene polymorphism and the serum 25-hydroxy vitamin D (25-OHD) levels in COPD
patients (n=50) and healthy controls (n=50) from the north Indian population. The results revealed that there was a significantly (p=0.03) higher frequency of GC1 F allele in COPD patients than that in the controls. The 1F-1F genotype was observed to be contributing significantly to COPD. The levels of 25-OHD were significantly (p=0.045) lower in the COPD patients in comparison to the controls. Also these levels were observed to be lowest in GC 1F-1F individuals.

Moussa et al. (2014), in a case-control study comprising COPD (n=16, 48.99±5.33y) and non-COPD (n=29, 47.93±5.66y) groups with similar smoking patterns, assessed the levels of MDA, protein-cys-SH (PSH) and GSH. There was prevalence of various health effects viz. cough (25.0vs.37.90%), sputum (37.50vs.34.50%), dyspnea (50.0vs12.40%), chest pain (12.50 vs. 20.70%), whistling (6.20 vs. 10.30%), recent respiratory infection (12.50 vs. 0.00%) and high blood pressure (18.50 vs. 6.90%), in both the COPD and the non-COPD groups. The values of FEV1, FVC and FEV1/FVC were decreased in COPD group compared to those in the non-COPD group. The COPD group also had significantly lower levels of GSH and PSH while no significant differences were observed for the levels of MDA.

Pawar et al. (2014) in a case-control study comprising 195 COPD patients (Stage I n=33; Stage II COPD n=52; Stage III n=57; Stage IV n=43) and 60 healthy controls reported that the serum MDA and nitric oxide levels were increased significantly with increased disease-severity and all COPD patients had increased MDA levels in comparison to the controls whereas the vitamin C, vitamin E and GSH decreased significantly in all COPD stages compared to the control levels. Correlation analysis revealed negative correlation between MDA and FEV1 per cent in COPD patients whereas the vitamin C levels showed a positive correlation with FEV1 per cent predicted in stage III and stage IV COPD patients, also of vitamin E with FEV1 per cent predicted in stage IV COPD patients and reduced glutathione, and FEV1 per cent predicted in stages II, III and IV COPD patients.

Waseem et al. (2014) assessed MDA, catalase, glutathione peroxidase and Superoxide Dismutase levels in 73 COPD patients (48 male and 25 females) categorized into mild (n=32) and moderate (n=41) groups. The results of the study revealed that the estimated values of GPX, SOD and Catalase were significantly (p<0.001) lower
whereas the MDA levels were higher in Moderate COPD group in comparison to levels in the mild COPD group. A significant inverse correlation of MDA levels was observed with SOD (p=0.01), catalase (p=0.01), GPX (p=0.01), FEV\textsubscript{1} (p=0.05) and per cent FEV\textsubscript{1} Predicted (p=0.01).

2.10 DNA damage, Oxidative stress and COPD

Assessment of both, genetic damage and oxidative stress, in COPD patients has only been carried out by Ceylan et al. (2006). The authors assessed DNA damage (by the alkaline comet assay) in moderate COPD patients, other having a history of smoking (n=47; >20 pack years) or of biomass cooking-exposures (n=25). The COPD patients from biomass cooking-exposure comprised 25 non-smoker women with 2–4h per day (since 10 years) of exposure to biomass-cooking. The control group comprised healthy non-smokers (n=36) with normal lung function. The results of the study revealed that the DNA strand-breaks (p<0.001), MDA (p<0.05) and protein carbonyl (p<0.05) levels were significantly higher in COPD patients in comparison to the controls. DNA damage was significantly (p<0.05) higher in COPD patients with smoking habits in comparison to those exposed to biomass-cooking, though no difference was observed between the two groups of patients for MDA and protein carbonyl levels. Also, DNA damage showed positive correlation with MDA levels in the smoking-related COPD patients. The authors suggested that oxidative stress (which is one of the main pathogenetic components of the airways’ inflammation in COPD) caused the observed genetic damage.

2.11 Oxidative stress, Lipid Levels and COPD

Only a single study (Waseem et al., 2014) has assessed the oxidant-antioxidant imbalance and lipid profile in exercising (n=50) and non-exercising (n=40) moderate COPD patients. The spirometric values of FVC, FEV\textsubscript{1}, FEV\textsubscript{1}/FVC and FEV\textsubscript{1} per cent predicted were significantly (p<0.001) lower in the non-exercising group. The levels of superoxide dismutase were also significantly (p<0.001) decreased by 1.07 folds, catalase by 1.11 folds and glutathione peroxidase by 1.05 folds whereas the MDA levels were significantly (p<0.01) increased by 1.05 folds in the non-exercising group in comparison to the exercising group. A significant 1.09 fold (p=0.03) decrease in VLDL levels and 1.17 fold significant (p<0.001) increase in HDL levels were observed in the exercising group when compared to the non-exercising group though a non-significant
decrease was observed between the two groups for the values of TC, TG and LDL levels.

2.12 Genetic Polymorphisms and COPD

The differential metabolizing ability for exogenous and endogenous compounds is responsible for inter-individual variability, and therefore reduced genetically-determined detoxifying capacity has the potential to increase risk for adverse health effects vis-à-vis to those with unaltered metabolic capacity (Harness et al., 1991).

The manifestation of COPD results from the interaction of polygenic and environmental factors induced by an oxidative-stress response and protease imbalance under the influence of genetic variants (MacNee, 2005; Kumar et al., 2013). Therefore, for the present study, the metabolic genotypes of GSTT1, M1 and P1 and of the candidate gene, SERPINA1 were studied. Here under, one by one, first the background on the GST and SERPINA1 gene variants is presented and then genotyping studies in relation to COPD-risk are reviewed.

2.12.1 Glutathione-S-transferases (GSTs)

These are the phase-II metabolic enzymes that detoxify endogenous and exogenous substances (Laborde, 2010) by conjugating with the non-polar xenobiotic compounds with highly charged antioxidants to make it polar, thereby facilitating their excretion in the presence of phase III metabolic enzymes (Hayes et al., 2005; Josephy, 2010). The GSTs are cytosolic, mitochondrial and membrane-bound microsomal enzymes (Liang et al., 2013).

GSTM1 - The glutathione-S-tranferase (mu) gene maps on the short arm of chromosome 1 (1p13.3) and has eight exons ranging in size from 36-112bp and seven introns ranging in size from 87-2641 (Roodi et al., 2004). A wide range of variation in GSTM1 homozygous deletion polymorphism (approximately 20–67%) has been observed globally with regard to various ethnicities. The null variant of GSTM1 is of particular interest, as a plethora of studies have demonstrated the difference in susceptibility, exposure to environmental toxicants, resistance to chemotherapy treatment, variability in drug response, manifestation of several diseases as well as cancer outcomes linked to this SNP (Bhattacharjee et al., 2013).
**GSTT1**- The gene for the theta isoform of the enzyme is located at the long arm of chromosome 22 (22q11.2). The gene consists of five exons ranging in size from 88-195bp and four introns ranging from 205-2363bp. The gene is predominantly expressed in erythrocytes (Hayes and Strange, 2000).

The *GST M1* and *T1* genes are polymorphic, with a phenotypic absence of enzyme activity because of the homozygous deletion of the respective genes i.e. the null genotypes (Seidegard *et al.*, 1988; Pemble *et al.*, 1994).

**GSTP1**- The *GSTP1* (11q13.3) is 2.8kb long and has seven exons and six introns and there are several genetic variants. The *GSTP1* (rs1695; 313A>G) is a common polymorphism resulting from A>G substitution at position 313 in exon 5 leading to the replacement of isoleucine (Ile) by valine (Val) at 105 amino acid (Ile105Val)position (Natis *et al.*, 2005). The homozygous GG genotype alters the protein function and diminishes the detoxification thereby having the potential to increase DNA damage and may also increase the risk of inflammatory-induced diseases (Dunna *et al.*, 2012).

2.13 Molecular genotypes of GST and COPD

Literature documentations on genotyping results of *GST* (*T1, M1 and P1*) in COPD patients are reviewed here and some inconsistency appears for the association of the genotypes with disease emphasizing ethnic disease pre-disposition.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of *GST* polymorphisms in COPD patients (n=53) and controls (n=50) from Tokyo (Ishii *et al.*, 1999) revealed higher frequency of *GSTP1* (Ile105Val) homozygous wild types (Ile/Ile) in cases compared to the controls. It was suggested that the *GSTP1* Ile/Ile genotype might be less protective against xenobiotics in tobacco smoke, and hence showed association with COPD.

Genotyping of 83 COPD patients and 76 healthy controls (Yim *et al.*, 2000) for *mEPHx, GSTM1* and *GSTT1* genes revealed that these genes and their combinations did not show association with COPD in Korean cases.

However, in a study from Taipei, Taiwan (Cheng *et al.*, 2004) on 184 COPD patients and 212 controls, significantly (p=0.000) higher frequency of the *GSTM1* null genotype
was reported in patients as also of significant (p<0.001) association of the mutant \textit{mEPHx} exon 3 allele (histidine 113), \textit{GSTM1} null and homozygous Ile105Ile of \textit{GSTP1} combination. Severe COPD cases, compared to moderate cases, had higher frequency of \textit{mEPHX} exon 3 allele and the \textit{GSTM1} null genotype.

Gaspar \textit{et al}. (2004) in a Brazilian population of European ancestry reported a significant (p<0.01) four-fold increased risk for COPD in individuals with heterozygous (Ile/Val) \textit{GSTP1} and the \textit{GSTT1} null genotypes. However among the Chinese from Hong Kong and Southern China (Chan-Yeung \textit{et al}.., 2007), neither of the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} genotypes showed an association with COPD. Although in a case-control study on a Bulgarian population, Dimov \textit{et al}. (2008) reported a significant increase in the frequency of \textit{GSTM1} null (p=0.003) genotype in COPD patients, conferring a 3.60 fold increased risk for the disease.

In COPD patients (n=217) and healthy controls (n=160) from the Slovak population, Židzik \textit{et al}. (2008) observed that the \textit{GSTM1} and its combination with \textit{EPHX1}, His113-His113 conferred an increased risk for COPD, both before and after adjustment for age, gender and smoking status.

In a study on Tunisian COPD patients by Lakhdar \textit{et al}. (2010), a significant (p=0.0013) association of the homozygous mutant allele (Val/Val) of \textit{GSTP1} gene (exon 5; Ile 105 Val) was reported.

Genotyping of \textit{GSTT1}, \textit{GSTM1} and \textit{HMOX-1} genes in COPD cases (n=250) and controls (n=250) from Telangana, India (Begum \textit{et al}.., 2014), revealed a significant association of the \textit{GSTM1} null genotype (p=0.000) and of the GC genotype of \textit{HMOX-1} gene (p=0.05) with COPD.

Among the isocyanate-exposed persons in Bhopal, India, Bose and Bathri (2012) had reported that the homozygous (Val/Val) and heterozygous (Ile/Val) \textit{GSTP1} genotypes showed significant associated with COPD (n=23) though \textit{GSTT1} and \textit{GSTM1} null genotypes did not show any association.

In COPD (n=204) and lung cancer (n=218) patients from North India, Shukla \textit{et al}. (2013) had observed significant association of \textit{GSTM1} null genotype with COPD and of \textit{GSTT1} null genotype with lung cancer. Dey \textit{et al}. (2014) have also documented
significant association of GSTM1 null genotype but not of GSTT1, in COPD patients living in proximity to an open-cast coal mine area at Ledo, Assam.

Male caucasian Croatian COPD cases (n=30) and controls (n=60) showed significant association with both, GSTM1 and GSTT1 null genotypes and COPD as well as of the homozygous (GG) mutant of GSTP1 (exon5) and of the homozygous mutant (TT) and heterozygous (CT) genotypes of GSTP1 (exon 6) in a study carried out by Zuntar et al. (2014).

In Serbian COPD patients (n=122) and controls (n=100), Stankovic et al. (2015) have recently revealed a significant association of the GSTM1 null variant (p=0.042) and of the GSTM1 null and GSTP1 homozygous (Val/Val) combination with COPD.

2.14 Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 1 (SERPINA1)

The SERPINA1 gene (14q32.1) covering 12.2 kb, has four coding exons, three untranslated exons and six introns (da Silva, 2014). The protein is multifunctional, combining its inhibitory properties with immuno-modulatory and anti-inflammatory activities against neutrophils, lymphocytes, macrophages, monocytes, mast cells and epithelial cells (Bergin et al., 2012). The lungs are the principal sites of protein-activity, where protection is provided to the connective tissue of the lower respiratory tract from proteolysis triggered by neutrophils during inflammation (Bals and Kohnlein, 2009).

Alpha-1-antitrypsin (AAT) deficiency (AATD) is an autosomal-codominant disorder characterized by reduced serum AAT protein levels with a significantly higher risk or developing pulmonary diseases, such as COPD, because of the uncontrolled activity of neutrophil elastase in the lungs (da Silva, 2014). More than 125 gene variants of SERPINA1 have been identified with abnormal protein plasma levels and the alleles may be ‘deficient’, reduced or null alleles (Liusetti and Seersholm, 2004).

For the present study four SNPs of SERPINA1MI- rs17580;Ala213Val;264A>T, M2-rs289294;Ala101His;342G>A, S- rs6647; Glu264Val; 213C>T, Z- rs709932; Glu342Lys; 101G>A, were genotyped.

2.14.1 SERPINA1 Gene Polymorphism and COPD
The studies investigating the association of \textit{SERPINA1} with COPD are reviewed. Sandford \textit{et al.} (2001) genotyped \(\alpha_1\)-antitrypsin deficiency Z allele (multiplex-PCR analysis) in 283 individuals with low lung function and in 308 with normal lung function. Low FEV\(_1\) showed significant (p=0.03) association with the MZ genotype and a stronger one (p<0.05) for the combination of family history (first degree relative) of COPD with the MZ genotype.

In COPD patients (n=89) and controls (n=112) from Korea, Cha \textit{et al.}(2008) documented a significant association (p<0.05) of M1Val/M1Val, M1Val/M2 and M2/M2 genotypes with increased risk for COPD. The authors suggested that the lower elastase activity because of the M2 variant predisposes the individuals to more risk than in those with the M1 Val variant.

However in a case-control study, Denden \textit{et al.} (2010) observed no significant differences in the frequencies of \textit{SERPINA1} variants (M1 (Ala 213Val), M2 (Arg101His), M3 (Glu376Asp), S (Glu 264 Val) and Z (Glu342 Lys)) between COPD patients (n=100) and healthy controls (n=200) from Tunisia. Neither was there decline of FEV\(_1\) in COPD patients nor any association with these \textit{SERPINA1} variants.

In North Indian (Sobti \textit{et al.}, 2011) COPD cases (n=200) and controls (n=200), there were 5% MS \textit{SERPINA1} heterozygous genotypes. The M2 genotypes were also present in both cases (5.00%) and controls (1.50%) with 0.50% cases having the SS and 1.00% having the ZZ genotypes. The MZ genotype also exhibited association with obstruction even after correction for age, gender and smoking and an increased COPD-risk of 3.82 folds (p=0.03). Also the risk was 4.15 folds increased (p=0.05) in those with the heterozygous MZ and homozygous ZZ genotypes.

In a candidate gene-association study carried out in caucasians and African-Americans by Enewold \textit{et al.} (2012), the S and Z \textit{SERPNA1} gene variants and 11 tagging SNPs in the region of elastase 2 gene were studied for association with susceptibility to COPD and lung cancer. The study participants comprised patients with prevalent COPD (n=145), with non-small cell lung cancer (n=203) and with both, COPD and lung cancer (n=118), and 317 controls with no disease. The African-American, carriers of \textit{SERPINA1} S or Z had higher risk of having COPD as well as lung cancer.
Geramizadeh et al. (2013) in Iranian COPD patients (n=130) and controls (n=59) however reported that all participants with normal AAT levels had the MM genotype and there was lack of mutants for the S and Z alleles of SERPINA1 gene.

2.15 DNA damage, Genetic Polymorphisms and COPD

Only a single study of this type has come into attention. In COPD patients (n=51, 30males, 21females, 65.33±8.91y) treated at Santa Cruz Hospital, Brazil and age, gender and BMI- matched controls without pulmonary disease (n=51, males 28, females 23, 63.61±9.40y) from the white population, the role of genetic polymorphisms of XRCC1 (Arg399Gln), OGG1 (Ser326Cys), XRCC3 (Thr241Met) and XRCC4 (Ile401Thr) genes on modulation of DNA damage (comet assay and buccal micronucleus cytome (BMCyt) assay) and progression of COPD was assessed (da Silva et al., 2013b). There was a significant (p=0.000) decline in FEV1 per cent predicted (42.90±19.03 vs. 86.14±11.72) and FEV1/FVC per cent predicted (67.92±19.58 vs. 105.24±70.28) in controls compared to the patients and the patients had mild (n=8), moderate (n=16), severe (n=16) and very severe (n=11) COPD. The patients (n=36) and controls (n=9) had >30y of smoking duration. The smoking status in patients and the controls varied from never smokers (5 vs. 22), former smokers (34 vs. 25) and current smokers (12 vs. 4). The assessment of damage index using the alkaline comet assay (36.71±25.41 vs. 26.65 ±27.96, p=0.005), neutral comet assay (47.53±32.45 vs. 37.49 ±38.05, p=0.047) and the residual damage assessed by treatment of methyl methanesulphonate (MMS) (145.69±74.11 vs. 54.63 ±40.32, p=0.000) were significantly increased in COPD patients compared to the controls. No significant difference was observed for BMCyt assay as well as for the allelic frequency of the studied genetic polymorphisms between patients and the control groups. The DNA damage was significantly higher in COPD patients with the variant genetic polymorphisms of XRCC1 (Arg399Gln), XRCC3 (Thr241Met) in comparison to the control group. The correlation analysis revealed a significant positive correlation of the alkaline basal damage index with FEV1 and FVC in COPD patients. In contrast, negative correlation was observed between the basal DNA damage and per cent residual damage for deficiency in XRCC1 (Arg399Gln) repair. FEV1 and FVC correlated
negatively with the nuclear anomalies (BUD nuclear, binucleated, condensed chromatin and karyorrhectic cells) for both deficiencies in XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) repair.

### 2.16 Genetic Polymorphism and Genetic Damage

Studies on the genetic polymorphism of GST and CYP genes in persons with dust-exposure at workplace (in various related occupations as stone crushing) and/or smoking habits or with respiratory disease, and assessed for genetic damage are presented here under:

Chen et al. (2001) in a study on coke-oven workers (n=240) and controls (n=123) reported a significantly increased Olive tail moment in those with the AhR Arg554/Arg554 genotype though in controls, genetic damage was increased in those with the CYP1A1 Mst1 TT genotype compared to those with CYP1A1 Mst1 CC/CT genotype.

In a study comprising pesticide-exposed fruit growers (n=91) and healthy controls (n=106), Li et al. (2006) reported that tail moment was significantly higher in workers compared to the controls, and that a significant association of CYP3A5 and GSTP1 null genotypes with tail moment was observed.

Moretti et al. (2007) revealed significantly higher DNA damage in individuals exposed to PAH in a graphite electrode manufacturing plant (n=109) compared to the controls (n=82), while no significant differences were reported for the genetic variants of CYP1A1, EPHX and GSTM1.

da Silva et al. (2008) reported that only the PON genotype among others (GSTT1, GSTM1, GSTP1, CYP1A1, CYP2E1) showed association with micronucleated cell frequency in buccal cells and with damage index and damage frequency in peripheral blood lymphocytes in some agricultural workers.

Villarini et al. (2008) assessed primary and oxidative DNA damage, sister-chromatid exchanges (SCE) and micronuclei (MN) in under-ground road tunnel construction workers occupationally-exposed to dust, gases and diesel exhaust (n=39) and in healthy controls (n=34) along with genotyping for CYP1A1 and GSTM1 variants. The results
revealed significant increase in genetic damage in workers compared to the controls although there was no significant association of CYP1A1 and GSTM1 variants with genetic damage.

However, GSTM1 null genotypes showed significant association with higher levels of 8-OHdG as compared to the levels in those with GSTM1 present genotype (Lin et al., 2009) in 488 patients on haemodialysis.

Korean male smokers (n=49) with GSTT1 null genotype had higher levels of DNA damage and of conjugated dienes with decreased plasma HDL-C and atherogenic index as compared to those with the GSTT1 present genotypes (Lee et al., 2010).

Significantly higher (~13 times) DNA damage (comet assay) was present in the workers with CYP2D6*3 PM and PON1 (QQ and MM) genotypes (Singh et al., 2011) among the 150 workers occupationally-exposed to organophosphate pesticides compared to 134 healthy controls.

Singh et al. (2012) reported significantly increased DNA tail moment in occupationally-exposed organophosphate pesticide workers (n=134) having NAT2 slow acetylation and CYP2C9*3/*3 and GSTM1 null genotypes.

Significantly increased tail moment and buccal micronuclei (BMN) frequencies (Giri et al., 2012) were reported in coal-tar workers (n=115) in comparison to healthy controls (n=105) with significant influence of CYP1A1 M1 and the M2 heterozygous and homozygous variant genotypes on genetic damage.

Kadioglu et al. (2012) reported that in healthy individuals from Turkey (n=127), the GSTT1 null genotype had higher levels of chromosomal aberrations, micronucleated cell frequency as well as of single-strand DNA breaks compared to those with GSTT1 present genotype.

Investigating the genetic polymorphism of GSTT1, GSTM1 and GSTP1 in 65 infertile men with varicoceles and 30 healthy males from North-west China (Tang et al., 2012), sperm DNA damage (as assessed by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labelling; TUNEL assay, 8-OH-dG by HPLC) as well as levels of MDA and nitric oxide were significantly higher in those having GSTM1, GSTT1 and
GSTT1/GSTM1 null genotypes. Levels of TAC were also significantly reduced in these individuals compared to those with the present genotypes.

Wlodarczyk and Novicka (2012) investigated DNA damage in 220 healthy non-smokers in response to CYP1A1, GSTM1, GSTT1, GSTP1 and XPD genetic polymorphisms. Genetic damage observed as per cent DNA in tail was highest (6.70%) in those having the AA variant of XPD gene and the GSTM1 null genotype. The lowest levels (3.70%) were observed in GSTP1-AA/GSTM1(+) genotypes.

Urinary 8-OHdG levels in 148 non-smoker traffic policemen were significantly higher than in 135 normal controls (Prasad et al., 2013) and the authors also reported that the levels were increased significantly in those with the CYP1A1 M1 variant genotype and the null GSTM1 genotype.

Gomez-Martin et al. (2014) determined the levels of N-7 methyl deoxyguanosine as a biomarker of chemical methylating agent in 39 plastic green-house workers (exposed to low and high levels of pesticides), who were also genotyped for the paraoxonase-1(PON1) and GSTT1 and GSTM1 genes. Those with higher exposure to pesticides and with susceptible metabolic genotypes (individuals with GSTM1 null and PON1 192R) had higher DNA alkylation levels and higher risk of DNA damage.

Cho et al. (2015) assessed DNA damage and antioxidant status in 95 smokers (before and after grape juice-supplementation) depending upon glutathione-S-transferases genetic polymorphisms. In those with the GSTM1 null genotype, significantly decreased diastolic blood pressure, lymphocyte DNA damage and plasma-conjugated dines were observed. After a period of 8-weeks of grape juice-supplementation, all the DNA damage parameters (tail per cent DNA, tail length, tail moment) were significantly decreased in those having the GSTM1 null genotype. The tail per cent DNA and tail moment were both significantly decreased in the GSTM1 present genotype. In both, null and present GSTT1 genotypes, genetic damage decreased and antioxidant effect of grape-juice supplement was higher in GSTT1 present genotypes compared to the levels in GSTT1 null genotypes.

2.17 Stone-crushing/quarrying and Genomic Damage

There are only a few studies relating to genetic damage from exposure at the stone-crushing and related workplaces. These are reviewed here.
Sobti and Bhardwaj (1991) in workers (n=50) of a stone-crushing industry near Chandigarh reported a significant increase in (p<0.05) in frequency of chromosomal aberrations and sister-chromatid-exchanges in comparison to the values in controls (n=25).

A study on haematological and cytogenetic assessments in workers exposed to cement dust (Jude et al., 2002) revealed a significant (p<0.05) decrease in leucocyte count, platelet count and haemoglobin content in workers in comparison to the controls. Also a significant (p<0.05) increase in frequency of chromosome observations as well as sister-chromatid-exchanges and decreased meiotic index were observed in peripheral blood lymphocytes of workers in comparison to controls.

Male workers (n=58) comprising those involved in grinding and bagging (n=8) and outdoor- (n=17), indoor- (n=4) sand-blasters, and those from the glass-manufacturing industry (n=21) in Turkey (Demircigil et al., 2010), were assessed for micronucleus frequencies in peripheral blood lymphocytes and in nasal epithelial cells. Significant (p<0.001) increase in frequency of micronuclei in the peripheral blood lymphocytes (2x) and in nasal epithelial cells (3x) was observed in workers in comparison to the controls. The minerological assessments revealed a higher percentage of silica followed by aluminium oxide (Al₂O₃) and calcium oxide (CaO) in the sand-blasting dust-samples from the glass industry.

In a case-control study, Kaur and Gandhi (2011a) reported a significant (p<0.001) increase in the frequency of micronucleated cells in urothelial cells of workers (n=6) with 5-10 y of work-exposure at stone-crushing units located in Pathankot and Gurdaspur districts in comparison to the controls (n=5). Also in addition to decline in lung function (p<0.01) compared to controls, the workers had complaints of cough, shortness of breath, muscle/joint pains as well as redness and itching of eyes.

Kaur and Gandhi (2011b) in another study reported an elevated frequency of micronuclei in urothelial cells of workers (n=31) from five different stone-crushers situated in Pathankot (Baherian Bajurag village) in comparison to controls (n=19). Genetic damage showed significant association with age, workplace exposure and work hours at unit.
Quarry workers (n=45) with 10-15y of exposure history and involved in stone-crushing, grinding, carrying chips and extracting brick area were compared to the controls (n=20) for DNA damage in peripheral blood lymphocytes and frequency of micronuclei in buccal cells (Halder and De, 2012). Cough, common cold, fever, headaches and diarrhea were common complaints among the workers. Also observed in workers were significantly decreased Hb content (p<0.005) and significantly increased different grades (I, II, III and IV) of DNA damage (p<0.05) as well as micronuclei frequency (p<0.05) in comparison to the values in controls.

2.18 Stone-Crushing/Quarrying and Health Effects

The review on lung function decline and health effects from stone-crushing/quarrying activities is presented here.

Ghotkar et al. (1995) in a study on workers (n=80) from three quarries from Pachgoon area, Nagpur reported respirable-dust in high concentrations at stone-cutting (23.42hg/m$^3$), loading (20 hg/m$^3$) and crushing (15.38hg/m$^3$) sites. Male workers had a significant (p<0.05) decrease in peak expiratory flow rate (PEFR) and of FEV$_1$ and FVC compared to female workers. Decline in lung-function exhibited significant association with increased age, duration of dust exposure, smoking status and chronic obstructive airways disease.

A cross-sectional study on radiographic abnormalities indicative of pneumoconiosis in construction workers (n=1339) was conducted by Nij et al. (2003). The determined radiological abnormalities compared to median results of the International Labor Organisation (ILO) System revealed an abnormality of ILO profusion category 1/0 and greater in 10.20% of chest radiographs and profusion category of 1/1 or greater in 2.90% of the radiographs. These were attributable to increased exposure from quartz-containing dust at construction sites.

Tiwari et al. (2003) assessed the lung function parameters in quartz stone ex-workers (n=134) from Godhara, Gujarat and reported a decrease in FVC, FEV and PEFR values in females in comparison to values in males. These lung function parameters also decreased significantly (p<0.05) with increased duration of exposure.

In a first covert case of silicosis from India, copper levels were reported to be increased (Tiwari et al., 2005) in an asymptomatic 22y-old male worker at a quartz-crushing unit.
Chattopadhyay et al. (2006) compared the pulmonary test parameters of stone-crushing workers (n=272) and the agricultural workers (n=123) of West Bengal, India. The FVC and per cent FEV, values were significantly decreased (p<0.001) in agricultural-compared to the stone-crushing-workers although in the latter, these values decreased with increased duration of work.

Reddy et al. (2007) in workers (n=50) from stone-crushing units in Paraercherla (near Guntur) reported abnormal chest expansion and peak expiratory flow rate (PEFR) in comparison to workers from the ornamental industry.

The stone-crusher units (n=5) at Chitrakoot area, West Bangal had very high suspended particulate matter (SPM, 100-650 μg/m³), SO₂ (150-201 μg/m³) and NO₂ (300-750 μg/m³) values in comparison to the Indian permissible levels of 360 μg/m³ (for SPM) and 80 μg/m³ each for SO₂ and NO₂). Respiratory disease, cough, headaches, allergy and tiredness were the main complaints reported by the workers (n=35). Also the effect of dust on flora was assessed which revealed closure of stomata in plants at a distance of 100m from the crushers while those at 300m were opened maximally (Chaurasia et al., 2009).

Respiratory disease symptoms and skin dermatoses were the most prevalent complaints significantly (p<0.05) associated with dust-inhalation in quarry workers (n=270) at three quarries, as compared to controls (n=290) from Nigeria (Ugbogu et al., 2009). The composition of dust particles at quarries comprised Fe, Zn, Cd, Ni, Pb, Cr, Ba, Be and Al.

Ilyas and Rasheed (2010) reported that 14% of the deaths in stone-crushing workers (n=221) from Pakistan occurred from chest infection. The most prevalent health complaints were of eye infection and backache.

Jhony et al. (2011) in building-demolition workers (n=55) from India reported a significant (p<0.001) decrease in values of FVC, FEV₁ %FEV₁, %FVC, PEFR and FEF25-75% in comparison to values in controls (n=40).

Assessment (Ilahi et al., 2012) of parameters related to liver and kidney function revealed significantly (p<0.05) higher levels of glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), bilirubin and creatinine in workers (n=66) at some stone-
crushing units in Khyber Pakhtunkhwa, Pakistan, in comparison to the levels in controls (n=66).

A cross-sectional study on stone-crushing workers (n=287) from Ratua, Bhopal (Narkhede et al., 2012) reported musculoskeletal complaints, breathing problems and generalized weakness. The workers with work exposure of >5y had higher rates of respiratory problems in comparison to those with <5y of exposure.

Chest pain, cough, shortness of breath and wheezing were complained by quarry workers (n=403) from Ebonyi state, Nigeria (Nwibo et al., 2012). The study also revealed a significant decrease in FEV₁ and FVC values with increase in years of exposure of work at the quarry.

The respiratory status of construction- (n=101) and sanitary- (n=56) workers were compared with those of controls (n=92) from Thoothukudi, India (Mariammal et al., 2012). Respiratory complaints reported by both, construction and sanitary workers with >15y of work exposure, comprised dyspnea, sinusitis, sneezing, running nose and asthma, though the prevalence of these complaints was higher in sanitary- compared to that in construction-workers. However, the lung function parameter in both the construction and sanitary workers was significantly elevated (p<0.05) in comparison to that in controls.

Farhadian et al. (2013) in workers (n=117) employed in stone-crushing workshops in Hamadan province of Iran reported a decline in lung function parameters (p<0.01) workers in comparison to the controls (n=110).

Pulmonary function test assessment in male stone-crusher workers (n=120) from seven stone-crushers in Marathwada, Maharashtra (Rathod and Sorte, 2013) revealed that with increase in the duration of exposure from dust, there was a significant (p<0.001) decline in values of FVC, FEV₁, FEF₂₅₋₇₀%, PEFR and of maximal voluntary ventilation (MVV).

In a study comprising 50 (25 males; 25 females), stone-crushing unit workers, Chaurasia and Karan (2014) reported that at four units, the total suspended particulate matter (498-721 μg/m³) and the PM₁₀ (282-562 μg/m³) concentration exceeded the respective CPCB standard levels (200 μg/m³ and 100 μg/m³). The concentration of PM₂.₅ was in the range of 162-245 μg/m³ and the levels of FVC, FEV₁ and PEF were
lower than the predicted values of these indices. More than one-thirds of the workers reported complaints of cough, chest-pain, and chest tightness and difficulty in breathing.

A study comprising workers (n=120) from stone-crushing in Maharashtra (Rathod et al., 2014) revealed a significant decline in the values of FEF$_{25-75\%}$ and PEFR in comparison to those of controls (n=120). These values also decreased with increased duration of work at the crushers.

In a study comprising 75 each of male quarry workers and controls from Andhra Pradesh, India, there was a significant decline for FVC, FEV, FEV/FVC, %PEF and FEF 25-75% in workers in comparison to the controls (Kumar et al., 2014).

Socio-demographic variables and the health status of the workers (n=150; 138 male, 12 female) from ten stone-crushers in Yelakeli, Wardha were assessed by Nayak et al. (2014). The workers had <5y of work experience, and about 81% of the workers did not use any protective equipment. The lung function parameters were abnormal in 35% of the workers and musculoskeletal, eye and respiratory complaints were most prevalent among them.

Tolinggi et al. (2014) reported a significant increase in levels of serum IL-8 in workers (n=9) from lime-stone mining industry at Wangun village, Palang in comparison to the controls (n=9). However, there was no significant differences between the two groups for pulmonary function parameters.

A study on 80 workers (10 from each quarry) from eight quarries in the Nafusa mountains of Libya (Draid et al., 2015) reported a significant decline in the values of FEV, (p=0.003), FEV/FVC (p=0.009) and PEF (p=0.03) in comparison to values in controls (n=85).

The pulmonary function test assessment in stone-crushing workers (n=50) and controls (n=50) from Nagaur, Rajasthan (Mirdha et al., 2015) revealed highly significant (p≤0.001) decrease in FVC, FEV, FEV/FVC and PEFR values in workers in comparison to the controls.

Among 80% of workers (n=98) at stone-crushing units in Palestine (Rahhal et al., 2015), the prevalent complaints were of cough, sputum-production and chest pain with 69% of the workers having restrictive pattern, 67% mild restrictive and 11.2% had
obstructive pattern of lung function. Significant (p < 0.001) decrease in FVC values (p < 0.001) and increase in FEV/FVC values were observed in comparison to the predicted values.

Lung-function assessment in workers (n=100) from a stone quarry in Rayachoty, Kdapa (HP) by Shaik et al. (2015) revealed a highly significant (p=0.000) decrease in values of FVC, FEV, PEFR and per cent FVC in workers compared to the values in controls (n=50).

2.19 Genotoxicity of Elements present in Top-soil samples of stone-crushing units

A report by the Geological Survey of India (GSI, 2008-2009) on the geological analysis of the top soil of Punjab districts (including the ex-Gurdaspur district of which Pathankot was at that time a part where stone-crushing units are located and were sampled) has revealed the presence of silicon dioxide (SiO₂), ferric oxide (Fe₂O₃), Yttrium (Y), Zirconium (Zr), Rubidium (Rb), Strontium (Sr) and Barium (Ba). Various other stone-crushing units in India and abroad have primarily reported the presence of silica, quartz, oxides of aluminium, iron, magnesium and sodium, titanium dioxide, phosphorus pentoxide, lead, selenium, zinc, cadmium, chromium, nickel, beryllium, quartz, wollastonite, feldspar, pyroxene, biotite (Golbabaei et al., 2004; Sivacoumar et al., 2006; Ugbogu et al., 2009) depending on the rock/soil strata-type. In a study from Gurdaspur district, Sinha et al. (2007) had documented the highest presence of SiO₂ (49.26-87.52 wt%) compared to those of aluminium oxide (Al₂O₃), ferric oxide (Fe₂O₃), magnesium oxide (MgO), potassium oxide (K₂O), calcium oxide (CaO), sodium oxide (Na₂O) and titanium dioxide (TiO₂) in mud stones from river Ravi in Gurdaspur.

The soil-samples collected from the stone-crushing units in the present study contain Si,Al,Na,Fe,Mg, K,Ca,Cu as revealed by EDX-SEM analysis. As these elements have the potential for genotoxicity (Omar et al., 2012; Tchounwou et al., 2012), a review on the genotoxicity assessment of the elements from 2010 onwards is tabulated.

Table 1. Genotoxicity Assessment of Elements Revealed in Soil Samples of Gurdaspur and Pathankot Districts

<table>
<thead>
<tr>
<th>Reference</th>
<th>Biological System Exposed</th>
<th>Biomarker studied</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi et al.(2011)</td>
<td>Mammalian cells</td>
<td>Comet assay</td>
<td>2 fold increased tail</td>
</tr>
<tr>
<td>Reference</td>
<td>Biological System Exposed</td>
<td>Biomarker studied</td>
<td>Observation</td>
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<tr>
<td>Kang <em>et al.</em> (2011)</td>
<td>L5178Y Cell lines exposed to magnetic nanoparticle silica</td>
<td>Comet assay</td>
<td>Significantly increased DNA strand breaks</td>
</tr>
<tr>
<td>Mu <em>et al.</em> (2012)</td>
<td>T29, HaCat and A549 cells exposed to 0.1μg/ml and above</td>
<td>Comet assay</td>
<td>Tail moments differed at different concentrations in comparison to positive control (hydrogen peroxide)</td>
</tr>
<tr>
<td>Musa <em>et al.</em> (2012)</td>
<td>Human lung fibroblast cell line, MRC-5 exposed to 0.00005, 0.0009, and 0.1 g/ml of hydroxyapatite-silica</td>
<td>Comet assay</td>
<td>DNA strand breaks and chromosomal alterations</td>
</tr>
<tr>
<td>Duan <em>et al.</em> (2013)</td>
<td>Human umbilical vein endothelial cells (HUVECs) cells exposed to silica nanoparticles by intravenous administration</td>
<td>Cellular morphology, cell viability and lactate dehydrogenase (LDH) activity</td>
<td>G2/M arrest through the upregulation and downregulation of cyclin checkpoints</td>
</tr>
<tr>
<td>Guidi <em>et al.</em> (2013)</td>
<td>Murine macrophages (RAW264.7) and human epithelial lung (A549) cell lines exposed to 0, 5, 10, 20, 40 and 80 μg/cm² of silica powder</td>
<td>Comet assay and micronucleus test</td>
<td>Increase in DNA strand breaks and chromosomal alterations</td>
</tr>
<tr>
<td>Balamuralikrishnan <em>et al.</em> (2014)</td>
<td>Silica-exposed workers from pottery industry</td>
<td>Chromosomal aberrations (CA), micronucleus (MN) and DNA damage (comet assay) in the peripheral blood lymphocytes</td>
<td>Significant increase in the frequency of CA, MN and the total DNA damage</td>
</tr>
<tr>
<td>Tarantini <em>et al.</em> (2015)</td>
<td>Male Sprague Dawley rats exposed to 5, 10, or 20 mg/kg to Synthetic amorphous silica (SAS)</td>
<td>DNA strand breaks and Oxidative DNA damage with the alkaline and the (Fpg)-modified comet assays, Chromosomal damage by Micronucleus assay and MDA levels</td>
<td>Increased DNA strand breaks, oxidative DNA damage, chromosomal damage and lipid peroxidation levels reported</td>
</tr>
<tr>
<td>Decan <em>et al.</em> (2016)</td>
<td>Mouse lung epithelial (FE1) cells</td>
<td><em>Lac Z</em> mutant frequency analysis; Micronucleus assay; Reactive oxygen species (ROS) measurement</td>
<td>Increased micronucleus formation and oxidative stress and cell viability decreased</td>
</tr>
</tbody>
</table>

**Aluminium**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Biological System Exposed</th>
<th>Biomarker studied</th>
<th>Observation</th>
</tr>
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<tbody>
<tr>
<td>Balasubramanyam <em>et al.</em> (2009)</td>
<td>Rat peripheral blood cells</td>
<td>Comet assay and Micronucleus assay</td>
<td>Increased per cent DNA in tail and micronuclei</td>
</tr>
<tr>
<td>Murali <em>et al.</em> (2010)</td>
<td>Root cells of <em>Allium cepa</em></td>
<td>Micronucleus (MN), chromosome aberration (CA) or spindle aberration (SA) and comet assays</td>
<td>DNA damage and cell death reported</td>
</tr>
<tr>
<td>Virgilio <em>et al.</em> (2010)</td>
<td>Chinese Hamster Ovary (CHO-K1) cells</td>
<td>Sister-chromatid-exchanges (SCEs), micronucleus (MN) formation</td>
<td>MN and SCE frequencies were increased significantly</td>
</tr>
<tr>
<td>Türkez and Toğar, <em>Laurus nobilis</em> leaf</td>
<td>Sister-chromatid-exchanges</td>
<td>Significant increases in...</td>
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<td>Reference</td>
<td>Biological System Exposed</td>
<td>Biomarker studied</td>
<td>Observation</td>
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<tr>
<td>(2013)</td>
<td>extract</td>
<td>(SCEs) and chromosome aberration (CA) assays</td>
<td>both, SCE and CA frequencies in cultures treated with aluminium as compared to controls.</td>
</tr>
<tr>
<td>Pereira et al. (2013)</td>
<td>Embryonic zebrafish cells ZF4</td>
<td>Comet assay</td>
<td>Increased DNA double strand breaks</td>
</tr>
<tr>
<td>D’Souza et al. (2014)</td>
<td>Swiss albino mice</td>
<td>Micronucleus assay</td>
<td>Treatment of aluminium acetate induced MN formation even at low doses in bone marrow, male germ cells and fetal liver cells</td>
</tr>
<tr>
<td>Klingelfus et al. (2015)</td>
<td>Kidney tissue cells of the fish Rhamdia quelen exposed to aluminum sulfate</td>
<td>Cell cultures for Sister-Chromatid-exchanges (SCEs)</td>
<td>Increased sister chromatid exchanges observed.</td>
</tr>
<tr>
<td>Alarifi et al. (2013)</td>
<td>Human skin epidermal (HaCaT) cells</td>
<td>DNA damage, oxidative stress and caspase-3 activity</td>
<td>Decreased cell viability and glutathione levels and increased lipid peroxidation, apoptosis and necrosis</td>
</tr>
<tr>
<td>Georgieva et al. (2013)</td>
<td>Rabbits</td>
<td>Chromosome aberration (CA) and sister chromatid exchanges (SCEs)</td>
<td>Significantly increased chromatid fragments and total aberrations</td>
</tr>
<tr>
<td>Gomes et al. (2013)</td>
<td>Hemolymph cells mussels <em>Mytilus galloprovincialis</em></td>
<td>Comet assay</td>
<td>Increased DNA strand breaks</td>
</tr>
<tr>
<td>Allaam et al. (2015)</td>
<td>Male goats</td>
<td>Chromosomal analysis and comet assay</td>
<td>Significant increase in numerical abnormalities as hypoploidy and polyploidy</td>
</tr>
<tr>
<td>Prá et al. (2011)</td>
<td>Healthy children and adolescents</td>
<td>Alkaline comet assay and Cytokinesis-block micronucleus assay</td>
<td>Significant increase in primary DNA damage and micronuclei and nucleoplasmic bridges</td>
</tr>
<tr>
<td>Pöttler et al. (2015)</td>
<td>Human granulosa cells (HLG-5) exposure</td>
<td>Micronucleus test</td>
<td>Significantly increased micronuclei</td>
</tr>
<tr>
<td>Sonmez et al. (2016)</td>
<td>Human whole blood cultures exposure</td>
<td>3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays; total antioxidant capacity (TAC) and total oxidant status (TOS); sister chromatid exchanges (SCEs), micronuclei (MN) and chromosome aberration (CA) assays and 8-oxo-2-</td>
<td>Significantly increased TOS levels, MN, SCE and CA rates and 8-OH-dG levels and decreased TAC levels</td>
</tr>
<tr>
<td>Reference</td>
<td>Biological System Exposed</td>
<td>Biomarker studied</td>
<td>Observation</td>
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<tr>
<td>deoxyguanosine (8-OH-dG) levels</td>
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<td><strong>Sodium</strong></td>
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<tr>
<td>Zengin et al. (2011)</td>
<td>Cultured human peripheral lymphocytes</td>
<td>Chromosomal aberrations (CA), sister-chromatid-exchanges (SCEs), and micronuclei (MN)</td>
<td>Significant increase was observed of CA, SCEs, and MN and decreased mitotic index</td>
</tr>
<tr>
<td>Mamur et al. (2012)</td>
<td>Human lymphocytes</td>
<td>Chromosome aberrations (CA), sister-chromatid-exchanges (SCEs), and micronucleus (MN)</td>
<td>Increased CA, SCEs and MN frequency at all concentrations</td>
</tr>
<tr>
<td>Basu et al. (2013)</td>
<td>Human lymphocytes</td>
<td>Trypan blue dye exclusion test and resazurin test, comet assay</td>
<td>Significantly decreased cell viability and increased genetic damage</td>
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<tr>
<td><strong>Magnesium</strong></td>
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<tr>
<td>Di Virgilio et al. (2011)</td>
<td>Rat osteosarcoma UMR106 cells</td>
<td>Micronucleus (MN) test and Comet assay</td>
<td>Significant increase in MN frequencies and DNA damage</td>
</tr>
<tr>
<td>Petrović et al. (2016)</td>
<td>Peripheral blood lymphocytes of rugby players</td>
<td>Comet assay</td>
<td>Four-week-long magnesium supplementation significantly decreased the number of cells with DNA damage,</td>
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<tr>
<td><strong>Silver</strong></td>
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<tr>
<td>Ghosh et al. (2012)</td>
<td>Swiss albino male mice, <em>Allium cepa</em> and <em>Nicotiana tabacum</em></td>
<td>DNA damage and chromosomal damage</td>
<td>Increase in the frequency of aberrant cells and per cent tail DNA percent</td>
</tr>
<tr>
<td>Li et al. (2012)</td>
<td><em>Salmonella</em> strains (TA102, TA100, TA1537, TA98, and TA1535)</td>
<td>Ames test</td>
<td>Significant increased frequency of micronuclei observed</td>
</tr>
<tr>
<td>Xu et al. (2012)</td>
<td><em>HeLa</em> cells</td>
<td>Cytokinesis-block micronucleus (CBMN), DNA microarray and GO pathway analysis</td>
<td>Significantly increased DNA damage and apoptosis, thousands of genes were up- or down-regulated</td>
</tr>
</tbody>
</table>

After the thorough perusal of the various documentations as available in literature and identifying the lacunae, the present comprehensive study was therefore planned to investigate the different dimensions of Chronic Obstructive Pulmonary Disease on a same set of cases identified at workplace by undertaking biochemical, genetic damage and molecular genetic analyses supported by physiological and anthropometric aspects and geo-chemical investigations.