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Oxidative DNA Damage, Oxidative Stress and Genetic Susceptibility-Prognostic Scores in 'Missing' COPD Cases

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ABSTRACT *In situ* identification of COPD cases at workplace and sample assessment for oxidative stress/ DNA damage as a function of metabolic/antioxidant genotypes (as susceptibility genotypes) offers a novel manner of hazardous workplace-identification for genotoxic/carcinogenic events. In this case-control study, blood/ sera samples of the COPD cases (n=32 identified spirometrically among the stone-crushing workers) and healthy controls (n=19) were assessed for 8-OHdG (DNA damage), glutathione and superoxide dismutase levels (oxidative stress) and genotyped for *GSTT1, M1, P1* and *MnSOD* variants. Significant increase in oxidative damage and lung-function decline were observed as a function of some genotypes. Predictors of genetic damage included tGSH, SOD and *GSTM1*. A prognostic index score based on prognostic factors was developed revealing cases were at high- (53.12%), intermediate- (34.37%) or low- (12.50%) risk for progressive DNA damage. These aberrant findings imply workplace exposure. This study provides insight on exposure-effect relationship in workers at stone-crushing sites.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is an obstructive airway disorder characterized by the slowly progressive and irreversible decrease in forced expiratory volume in one second (FEV_1), accompanied by emphysema and chronic bronchitis (Rabe et al. 2007; Cazzola et al. 2015); it develops with decreasing lung function as a function of age in the normal population (Ito and Barnes 2009). Despite the heritability of 40-77 percent, a host of other influences can also exacerbate this condition (Young et al. 2009). Rather the development and progression of COPD involves multiple genes, gene-gene and gene-environment interactions (•idzik et al. 2008; de Jong et al. 2015). Smoking exposure is considered as the most important risk factor for the development of COPD (Chan-Yeung et al. 2007; Kurmi et al.2015) with mild and moderate COPD cases having a three-fold risk of developing lung cancer within ten years, which increases to a ten-fold risk with severe COPD compared to the smokers with normal lung function (El-Zein et al. 2012). There also however exists COPD-associated increased mortality from lung cancer in non-ever smokers (Turner et al. 2007; Kiri et al. 2010; Aldrich et al.2015).

Despite quarrying and mining activities as important occupational set-ups inducing COPD (Jhoncy et al. 2011; Iftikhar et al. 2009), yet workplace identification of this occupational disease has not come to attention on pursuing literature related to COPD. Also there is uncertainty in prognosis of COPD although a number of validated indices exist (Briggs et al. 2008). As these indices require in-puts from patients and the interpretation by the care-giver/physician, these may not be able to cater to disease-identification in the field. In the present study, the on-site disease-identification using the recommended spirometry evaluation (Briggs et al. 2008; Shiota et al. 2015) to recognize COPD cases at stone-crushing units (dust exposure) was carried out. Such an identification at the workplace gains importance as 50-80 percent of COPD are missed-out on the basis of misdiagnosis/co-current diagnosis due to relying 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).

The effect of various environmental stressors from occupational exposures needs to be assessed for prediction of cancer outcome(s) (Fenech 2002) as 90 percent of cancer is environmental in origin (Hemminki et al. 2006). COPD has also been identified as an independent risk

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factor for lung cancer with inflammation as the pathophysiologic factor for high risk of its progression (Sin et al. 2006; Hillas et al.2015) and smoking- induced COPD associated with lung cancer has also been documented (Koshiol et al. 2009). While mechanisms governing the risk of developing neoplastic disease are not well known (Barreiro 2008), of the various theories, oxidative stress may be playing a pivotal role in its manifestation. In COPD, oxidative stress has been observed as ensuing from decreased FEV_1 (Kluchová et al. 2007), substantial inflammatory response increasing cytokines as triggered by exogenous dust particles (Yang et al. 2011) and decreased antioxidants because of depletions of glutathione peroxidase, superoxide dismutase (SOD), catalase, ascorbic acid and vitamin E (Borm et al. 2004).

The impaired oxidant-antioxidant status can cause cellular damage; DNA damage can result from the action of reactive oxygen species (Jackson and Loeb, 2001) and is the underlying cause of mutations leading to cancer (Bernstein, 2012).Chromosomal damage (increased micronuclei frequency) in peripheral blood lymphocytes has been extensively used for predicting risk of cancer (Fenech et al. 2011) and oxidative DNA damage is also implicated in carcinogenesis, ageing and age-related neurodegenerative diseases (Fortini et al. 2003, Nishigori et al. 2004). The major form of oxidative DNA damage is 8-hydroxy-2‘-deoxyguanosine (8-OHdG) resulting from G→T and A→C base substitutions. It may lead to mutagenesis if unrepaired and is directly correlated with lung carcinogenesis (Gackowski et al. 2003). The lesion 8-OHdG is an established biomarker of oxidative stress/oxidative DNA damage and being potentially mutagenic, it is useful as an intermediate marker of a disease end-point like cancer (Cheng et al. 1992). Therefore in the present study, 8-OHdG level was assessed as a pre-lesion of neoplasia in peripheral blood leukocytes (PBL) of COPD- identified cases at stone-crushing units. Although the leukocytes are not the direct target of the exposure at this workplace, they may possibly be affected by the accumulated unmetabolized toxic compound(s) in the lung (Gackowski et al. 2003). This hence prompted the assessment of oxidative DNA damage in the peripheral blood leukocytes and also because of the non- accessibility of the target (lung) cells.

The biomarkers of exposure and effect, and clinical disease (cancer) may further be influenced by susceptibility genotypes and their gene products as pre-dispositional factors (Their et al. 2003). Also as DNA damage and DNA repair have a major role in carcinogenesis and from occupational settings, the susceptible metabolic genotypes (gene products) may inherently be associated in causing genetic damage. Therefore genotyping of the occupational workforce was carried out for glutathione-S-transferase (*GST*) gene variants (both for disease susceptibility and genetic damage) since *GST* alleles have been documented to have an association with COPD (Young et al. 2011b). Furthermore, a reduced expression of these alleles has also been observed in the air passage of COPD patients (Imboden et al. 2007; Lakhdar et al. 2011) and hence the expression of glutathione-s-transferases was also estimated. Association of the *Val/Ala* variants of manganese superoxide dismutase (*MnSOD*) with lung cancer (Wang et al. 2001) further justified the genotyping of this allele and assessing its expression. Variant forms of these susceptible genes are generally common in the population. Due to their specificities for substrates they interact with during environmental exposures, they can increase the risk for disease-causation (Lan et al. 2000). Incidentally, *GST* and SOD enzymes are also involved in the metabolic and oxidative stress pathways (Borm et al. 2004), and since stone-crushing is an inflammation-triggering occupation (Vallyathan et al. 1995), the assessment of the amounts of these enzymes was thought appropriate.

The purpose of the present study was two-fold. On one hand to identify COPD cases from workplace exposure (occupation-related disease) and hence assist in identifying ‘missing’ COPD cases using recommended (spirometry) measurements (Briggs et al. 2008; Young et al. 2011a) and COPD categorizations (GOLD 2003). The other (main) purpose was to determine the propensity (Prognostic Index/score) for genetic damage and by extension an increased likelihood for carcinogenesis as ensuing from the combined effects/interactions of prognostic (risk) factors in COPD cases (the workers exposed to industrial-type prevalent conditions) at stone-crushing units. This entailed the evaluation for the presence of oxidative stress (GSH and SOD) and oxidative DNA damage in workers at stone-crushing units genotyped for the *GST* and *MnSOD* genes.

Objective

The objective of the present study was to assess oxidative DNA damage (8-OHdG) and oxidative stress (glutathione and superoxide dismutase) alongwith molecular genotyping of *GSTT1*, *GSTM1*, *GSTP1* and *MnSOD* gene variants in COPD cases identified from stone-crushing units and in healthy controls.

MATERIAL AND METHODS

Study Design

The present study was carried out after obtaining approval from the Institutional Ethics Committee. In order to investigate for genetic damage from occupational exposures as from stone-crushing activities, villages near Pathankot (Sarna, Mirthal) and Gurdaspur (Babbehali) of Punjab, India where four stone-crusher units are located, were visited. The workforce comprised local Mazhbi Sikh and Scheduled Caste and Backward Class Hindu males primarily engaged in drilling, dressing and loading of stones (all dust-emanating activities) with an occupational exposure from five to 14 years. Most of the workers were staying on-site. No preventive or precautionary measures, as set-up in the guidelines by the government (CPCB 2009), were prevailing.

Group discussions were held with the workers and after voluntary written informed consent, their demographic details, medical, genetic-and exposure-histories and information on their life-styles and dietary habits were recorded on a pre-designed questionnaire. Healthy male subjects with no occupational/incidental or accidental exposure(s), of similar ethnic and geographic origin, comprised the control group. Since many of the workers as well as the controls were smokers, the Brinkman index for smokers was calculated by multiplying the number of *bidis* (Indian cigarette) smoked daily with the number of years of smoking, that is, smoking years (Kojima et al. 2005).The higher the Brinkman index, more are the *bidis* smoked for longer period of time (years). Anthropometric measurements for height and weight were also taken for each subject using standard protocols (Weiner and Lourie, 1981) so as to calculate the body mass index (BMI, kg/m^2) of the study participants.

The Pulmonary Function Test

The Pulmonary Function Test (PFT) is performed to assess lung function, lung damage, lung disease diagnosis and whether lung function is affected at work place/ occupational exposure (Jhoncy et al. 2011). The Spiro Excel spirometer model (Medicaid Systems, India) was used for carrying out the PFT. The subjects were asked to breathe in and out through the mouthpiece to get familiarized with the equipment. The Forced Expiratory Volume per second (FEV_1; l) and Forced Vital capacity (FVC; l) were recorded and the Forced Expiratory Ratio (FEV_1/FVC) calculated. The value of body surface area (BSA, m^2) was also recorded. The same instrument was used throughout the study and three successive readings were taken for each subject in a standing posture using the standard spirometric procedures. The best reading was used for calculation purposes as per the manufacturer's recommendations and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (GOLD, 2003). The best reading is when the flow curve (flow rate against volume of air exhaled) displayed is proper, without any artifact such as improper blow of air or leak from mouth piece and without coughing or closed glottis.

Identification of COPD Cases

The FVC values are decreased in pulmonary obstruction, emphysema, pleural effusion, etc, and FEV_1 is low in obstructive lung disease and reduced lung volume. In fact, the FEV_1 decline is a convenient standard for identifying subjects with COPD and those exposed to environmental pollutants (Jhoncy et al. 2011). Therefore, based on the obtained values of FEV_1, FVC, FEV_1/FVC and the specific respiratory distress symptoms among the workers, they were categorized as those at-risk (FEV_1/FVC 0.7-0.8, $FEV_1 \geq 80$ percent predicted spirometry, cough, sputum production), mild (FEV_1/FVC<0.7, $FEV_1 \geq 80$ percent predicted; with or without cough, sputum production) or moderate (FEV_1/FVC<0.7, FEV_1 between 50 and 80 percent predicted; with or without cough, sputum production) COPD cases following the criteria recommended by the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2003). Since

spirometry-evaluation is recommended for COPD-identification, all these workers had varying stages of COPD and have been classified as COPD cases. Hence after disease-identification, this study design took the shape of a case-control study.

Sample size-Power Calculation

Given that a p=0.05 incremental increase in genetic damage is considered significant on Student's t-test analysis and taking the average base-line 8-OHdG levels of 0.21pg/ml (SD=0.39) obtained in a preliminary study of an ethnic-specific control group, the total sample size requirement for the study group was calculated (Sigma XL software) as 42 for a statistical power of 80 percent with α=0.05 (after (Albertini et al. 2000)). Hence, the sample size of 51 of the present study is justified at the required power of statistical analysis at 95 percent confidence interval.

Oxidative DNA Damage Assessment

Serum levels of the 8-OHdG as the biomarker of oxidative damage was measured with the highly sensitive enzyme-linked immunosorbent assay (ELISA) using the EIA kit (Stress Marq Biosciencec Inc, India). In tissues where this lesion is expected to have low levels (blood serum in the present study), the competitive *in vitro* ELISA is used. This technique is a valid and simple alternative tool to measure oxidative DNA damage with HPLC-EC or GC-MS techniques since a good correlation between ELISA and these methods has been obtained. The measurement of 8-OHdG in serum samples is reflective of overall oxidative damage in the body similar to that excreted in urine and includes damaged nuclear DNA (removed during base excision repair) as well as the damaged mitochondrial DNA and damaged DNA from the cytoplasmic nucleotide pools (Forlenza and Miller 2006).

For the present study, the manufacturer's instructions as given with the kit were followed. The serum samples were centrifuged, the samples and standards were added to 8-OHdG-coated microtitre plates followed by the addition of the 8-OHdG monoclonal antibodies. After an overnight incubation at 4 C, the plates were washed with a washing buffer, and a second enzyme-labeled antibody was added to each well. The chromogen was added after another washing and the contents were allowed to incubate in the dark for 15min. The subsequent colour development was observed; it is proportional to the amount of antibody, being inversely related to the amount of 8-OHdG in the serum sample; low colour indicates higher amounts of 8-OHdG. The absorbance was read at 415 nm using a computerized ELISA reader (BioRad 680 XR, USA). Triplicate readings were taken and the results in pg/ml were recorded at the last absorbance.

Oxidative Stress Analysis

The levels of oxidative stress biomarkers (glutathione and superoxide dismutase) were determined from blood sera samples using standard protocols as described below.

Glutathione (GSH) and Glutathione Disulfide (GSSG) Assays

Glutathione exists in the reduced (GSH) and oxidized (GSSG) forms and an optimal GSH: GSSG ratio is critical for cell survival and for an adequate oxidant-antioxidant balance (Gherghel et al. 2005).The total GSH (t-GSH) and GSSG levels were assessed using the GSSGR-DTNB (glutathione disulphide reductase-5, 5 dithiobis-2-nitrobenzoic acid)-recycling procedure (Rahman et al. 2007). In brief, for obtaining the standard curves for GSH and GSSG by linear regression, 0.50 nanomole increments (0-2nmoles) using GSH solution for the GSH and 0.025 nanomole increments (0-0.25 nmoles) for the GSSG assays, were prepared and their absorbance recorded at 412nm and 410nm, respectively on an ELISA reader (BioRad 680XR, USA). After adjusting with a blank, GSH and GSSG values were obtained in nmoles. Both, the GSH levels (GSH=t GSH-GSSG) and the redox index (ratio of GSH to GSSG) were calculated (Gherghel et al. 2005).

Glutathione (GSH) is a tripeptide thiol antioxidant and its intracellular concentration is an indicator of oxidative stress (Lushchak 2012). GSH neutralizes reactive oxygen species (ROS) and other radicals by detoxification involving the glutathione peroxidase (GPx), glutathione-S-transferase (*GST*) and glutathione reductase (GR). Normally in the cell, the ratio of GSH (reduced)/GSSG (oxidized) is 1:10 as GSH tends to be rapidly oxidized to GSSG with GSH ranging between 1-10 mM and GSSG representing <1 percent of the total cytoplasmic glutathione pool (Rahman and Adcock 2006). Glutathione reduc-

tase, in the presence of NADPH, recycles GSSG to GSH (Rahman and Adcock 2006). Hence the total amount of glutathione (tGSH) in the sample represents the sum of reduced and oxidized glutathione (tGSH=GSH+2GSSG). In the cells, both the reduced (GSH) and oxidized (GSSG) forms exist and oxidative stress leads to a reduced GSH/GSSG ratio (the Redox Index).

Superoxide Dismutase (SOD)

Serum samples were mixed with NBT (nitroblue tetrazolium), tritonX-100 and hydroxylamine hydrochloride. The absorbance was recorded spectrophotometrically (Thermo Scientific, USA) at 560nm after calibration with Tris HCl (blank).The amount of SOD in the sample was calculated from the absorbance values and expressed as U/ml. SOD inhibits the reduction of NBT by superoxide generated from the peroxidation of hydroxylamine hydrochloride, and the inhibition of chromogenic reaction is measured (Kono et al. 1978). Superoxide anions assist in the regulation of oxidative stress as the free anions are the substrate for generation of ROS (Siedlinski et al. 2009).These anions are degraded by SOD to H_2O_2, which if not detoxified by GP_X, converts to the more toxic hydroxyl radical. Of the three forms of SOD, *MnSOD* (manganese superoxide dismutase) is expressed in the mitochondria and accounts for ~ 90 percent of total SOD activity (Mak et al. 2007).The Val-9Ala variant (rs 179972) arises from a T→C substitution. This causes the secondary structure of the protein to change and affects the transport of *MnSOD* into the mitochondria (Cai et al. 2004) and the Ala/Ala *MnSOD* has higher enzymatic activity (Siedlinski et al. 2009).

Genotyping for GST and MnSOD Polymorphisms

Genomic DNA was isolated from whole blood using a standard protocol (*Sambrook and Russell, 2001*). Four polymorphic markers were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)/ PCR method (Applied Biosystems, India). The *GSTT1* (rs 17856199) and *GSTM1* (rs 366631) genotypes were determined by a multiplex PCR method (Vettriselvi et al. 2006) with intron 3 of HLADRB1 gene sequence as an internal control. The amplified *GSTM1* gene fragment comprised 220bp and 450bp for *GSTT1* gene with which the internal control fragment of 796 bp was amplified. The absence of the amplified product was consistent with the null genotype. Genotyping of *GST*P1 (Ile/Val; rs 1695) was carried out by the PCR-RFLP method (Vedyakov and Tonevitskii 2006). An A→G transition in its exon 5 introduces an *Alw261* digestion site with 176bp representing the Ile/Ile genotype and 91bp and 85bp bands indicate the Val/Val genotype while the presence of all the three bands indicate heterozygosity (Ile/Val). The PCR-RFLP method was also employed for genotyping *MnSOD* (Val/Ala; rs179972*)* (Cai et al. 2004). A T→C transition in codon 9 of the gene produces the Val→Ala substitution. *NgoMIV* digested the PCR product (107bp) into two fragments 89bp and 18bp in case of Ala/Ala (rs 179972), whereas homozygosity for Val yielded no digested product and only the 107bp fragment.

Statistical Analysis

The variables under study showed normal distribution on testing. The Chi-square analysis and the Student's t-test were used to compare the categorical and continuous demographic and clinical characteristics, respectively in the studied population. The statistical analysis of differences in oxidative DNA damage and oxidative stress biomarkers between workers (COPD) and controls was carried out using the two-tailed Student's t-test. Gene frequencies were estimated by the gene counting method/ Hardy-Weinberg equation and genotypic distribution was tested by Chi-square analysis. Hardy-Weinberg equilibrium was evaluated by the Chi-square test for goodness of fit. Effect of single and combined variants on COPD and on DNA damage and oxidative stress in workers and controls was investigated using odds ratio (OR) at 95 percent Confidence Interval (CI). As the cases and controls were matched for age, socio-economic status, smoking habit, alcohol drinking and BMI, no adjustment for these parameters was done. Correlations between different variables, both for oxidative stress and genotypes with oxidative damage, were determined by the Pearson's correlation and multiple linear regression analyses. In order to determine whether any of the demographical and clinically-relevant variables could be predictors for genetic damage, the standard Cox proportional hazard model was em-

ployed. On the basis of the significant prognostic factors for each subject, a prognostic index was developed based on survival analysis (Hoster et al. 2008) using the equation: Prognostic index=β_{1x}+ β_{2y}+….. (where x and y, etc are the significant prognostic factors). The differences in the prognostic scores in COPD categories were analyzed using the Student's t-test. The odds ratio and Chi-square analyses were performed using Medcalc software package and all other analyses were carried out using the SPSS version 16.00 for Windows. A p value $\leq$0.05 was considered significant.

RESULTS AND DISCUSSION

As the assessment of a large number of variables adds to the significance of human biomonitoring studies because of the possibility of multiple comparisons (Ketelslegers et al. 2008), therefore in the present study, a multitude of end-points for genetic integrity, oxidative stress and genetic predisposition to COPD and genetic susceptibility to DNA damage in workers (engaged in crushing stones and related activities) have been studied. This is of significance as the cascade of genotoxic/carcinogenic events involves the associations between various genetic polymorphisms and intermediary molecular markers as well as individual susceptibility towards environmental and occupational carcinogens (Naccarati et al. 2006). Attempt has further been made to assimilate all the obtained observations and translate the results as factors into a prognostic index/score. The observations from the study reveal that both, oxidative DNA damage and oxidative stress levels, were significantly increased in all workers (COPD cases) compared to controls. Genotypic associations varied with respect to genetic damage and oxidative stress. Hereunder the observations on demographic and clinically-relevant variables are first described followed by the findings on DNA damage, oxidative stress, genotyping, their correlation analysis and the prognostic scores.

Demographic Variables

The study group (Table 1) included workers (n=32, mean 30.34±0.95y) of mixed Punjabi ethnicity (Mazbi Sikhs, Scheduled Caste and Backward Class Hindus) engaged at four stone-crushing units in the same occupation from five to 14 y (mean 7.75±0.42y), and healthy controls (n=19, mean 29.73±1.55y) with no workplace/incidental exposure(s). The participants were all males and matched for age, socio-economic status, tobacco chewing, smoking as well as alcohol drinking habits with low BMI. The BSA and abnormal pulmonary function were the significantly differing features in workers. All non-exposed participants had normal lung function and so formed the control group.

On-site COPD Identification

The lung function parameters, FEV_1 (p=0.007) FVC (p=0.000) and FEV_1/FVC (p=0.001), were significantly lowered in workers. These aberrant spirometry findings underpin the risk from occupational dust exposure and so, on the development of these lung-dysfunctional parameters. The workers were classified as those at-risk, mild and moderate COPD cases (GOLD 2003). On discoursing with these workers about their lung-function values, the workers disclosed that they had not sought any medical assistance for their respiratory problems (missing COPD cases) and considered these as a part of the occupational hazards. Various reported risk factors for respiratory distress and COPD include age, BMI, sex, low socio-economic status (SES) and smoking and poor diet (Koster et al. 2004; Jindal 2006; Bemt et al. 2010;Lee et al. 2015). There were 81.25 percent workers in the present study who were smokers with a Brinkman index (69.93±12.54, range 15-256) significantly (p$\leq$0.01) higher. An almost similar number (81.25%) were in the continuous habit of tobacco and lime chewing. Alcohol-drinking was also prevalent (65.62%) and SES of most of the cases was low (87.49%) and hence could be important contributing factors for onset of COPD (Eisner et al. 2011; Crighton et al. 2015). Low BMI is another causal factor for COPD and the present group had 22% COPD cases who were underweight (<18.00 kg/m^2) with the BSA also significantly (p=0.019) lowered.

Respiratory distress reported by the workers ranged from lung damage to lung disease in all probability from exposure to the dust-emanating activities. Inhaled dust can irritate airways, cause defective oxygen diffusion and lung function impairment during stone quarrying/ crushing (Ghotkar et al. 1995; Chattopadhyay et al. 2006; Draid et al. 2015). It can lead to tubercu-

Table1: Demographic and clinical characteristics of the study group

Characteristics	*COPD cases No. (%)*	*Controls No. (%)*	χ^2 *value*	*P value*
Age (y) Mean± S.E.M. (Range)	30.34±0.95(20-45)	29.73±1.55(23-48)	-	-
<30	15(46.87)	13(68.42)	1.45	0.228
>30	17(53.13)	06(31.57)		
Occupational exposure (y) mean±S.E.M. (range)	7.75±0.42(5-14)	-	-	-
Diet - Veg/ Non-veg (occasional)	16(50.00)/ 16(50.00)	12(63.15)/07(36.84)	0.38	0.530
Smoking habit -Yes/ No	26(81.25)/ 06(18.75)	11(57.89)/ 08(42.10)	2.19	0.130
†*Brinkman Index (bidis[b] smoked daily x smoking years)*	69.93±12.54	25.05±7.87[a]		**0.010**
<100	18(50.25)	10(52.63)	2.12	0.340
<200	06(18.75)	01(5.26)		
<300	02(6.25)	-		
Tobacco +lime chewing (continuous)-Yes/No	27(84.37)/ 05(15.62)	13(68.42)/ 06(31.57)	0.97	0.320
Alcohol drinking (750-900ml/week)- Yes/ None	21(65.62)/11(34.37)	10(52.63)/09(47.36)	0.38	0.530
[c]*Socio-economic Status*				
Upper lower	13(40.62)	07(36.84)	0.32	0.850
Lower middle	15(46.87)	09(47.36)		
Upper middle	02(6.25)	02(10.52)		
[d]*Body Mass Index(kg/m^2)*				
<18.00 (underweight)	07(21.87)	-	11.12	**0.010**
18.00-22.90 (normal)	21(65.62)	10(52.63)		
23.00-24.90(overweight)	03(9.37)	04(21.05)		
>25.00(obese)	01(3.12)	05(26.31)		
Body Surface Area (m^2)	1.58±0.16	1.66±0.03[a]	-	**0.019**
[e]*Pulmonary Function test-COPD identification*				
Normal	-	19(100.00)	51.00	**0.0001**
At -risk	16(50.00)	-		
Mild	10(31.25)	-		
Moderate	06(18.75)	-		
††Ill-health Indicators(self-reported)				
Cough, Phlegm, Sputum	32(100.00)	-	-	-
Breathlessness	26(85.21)	-		
Eye irritation and watering	27(84.67)	-		
Skin irritation	21(65.62)	-		
Loss of appetite	17(53.12)	-		

Significant P values are in bold (p<0.05)

[a] Student's t-test;[b] Tobacco (0.15-0.25g) loosely packed in a hand –rolled, dried leaf of Tendu tree (*Diisopyros melanoxylon*), smoked as a cigarette. † (Kojima et al. 2005); [c] (Kumar et al. 2007); [d] (WHO 2004)/(Misra et al. 2009); [e](GOLD 2003);††Combinations with cough-phlegm-sputum indicators

losis, COPD, silicosis, emphysema, chronic bronchitis and laryngeal and lung cancers (Jhoncy et al. 2011; Iftikhar et al. 2009; Golbabaei et al. 2004; Tiwari et al. 2004; Sivacoumar et al. 2006). Respiratory and skin infections were observed in workers quarrying stones in a Nigerian study (Ugbogu et al. 2009), cough, dyspnea and wheezing in Peshawar (Pakistan) stone-crushing workers (Iftikhar et al. 2009) and respiratory distress including cough, phlegm and allergic disorders in those quarrying stones in Iran (Golbabaei et al. 2004). Pulmonary dysfunctions were also reported in individuals engaged in building-demolition (Jhoncy et al. 2011). These symptoms are triggering factors for COPD and are inclusive of the criteria for COPD categorization (GOLD 2003).

Oxidative DNA Damage

An approximately two-fold significantly (p=0.010) elevated oxidative DNA damage (Table 2)

Table 2: Oxidative DNA damage, oxidative stress and pulmonary function test in COPD cases and in non-exposed healthy controls

Variables		*COPD cases Mean ± S.E.M.*	*Controls Mean ± S.E.M.*	*P value*
Oxidative DNA damage	8-OHdG (pg/ml)	0.55**± 0.08	0.21± 0.09	**0.010**
Oxidative Stress biomarkers	tGSH(nmol)	215.91± 3.51	225.40*± 0.89	**0.046**
	GSSG(nmol)	26.80± 0.65	28.20± 0.78	0.187
	GSH(nmol)	189.10± 3.39	197.20± 0.96	0.077
	Redox Index	7.16± 0.21	7.09± 0.21	0.814
	SOD(U/M)	94.47± 1.37	99.36*± 1.33	**0.021**
Pulmonary Function Test parameters	FEV_1(l)	2.07± 0.24	2.99**± 0.09	**0.007**
	FVC(l)	3.05± 0.16	4.90***± 0.35	**0.000**
	FEV_1/FVC	0.57± 0.05	0.82***± 0.01	**0.001**

			COPD cases (Mean ±S.E.M.)				*Controls (Mean ±S.E.M.)*			
Disease Status		*N*	*8-OHdG (pg/ml)*	*tGSH (nmol)*	*SOD (U/M)*	*n*	*8-OHdG (pg/ml)*	*t GSH (nmol)*	*SOD (U/M)*
Controls	Normal	-	-	-	-	19	0.21±0.09	225.40*** ± 0.89^{*c}	99.36* ±1.33
†*COPD categories*	At-risk	16	0.58* ±0.13	218.56 ±1.70^{b}	95.54 ±1.97^{d}	-	-	-	-
	Mild	10	0.73** ±0.14^{b}	206.21 ±10.53^{c}	93.22 ±2.49^{e}	-	-	-	-
	Moderate	06	0.19 ±0.17^{b}	225.00 ±1.15^{b}	96.38 ±3.31	-	-	-	-

Values in bold are significant ($p \leq 0.05$), ***very highly significant ($p \leq 0.001$), **highly significant ($p \leq 0.01$), *significant ($p < 0.05$, Student's t-test) compared to controls; Values with similar letters are significant.
†(GOLD 2003); 8-OHdG-8-hydroxy-22 -deoxyguanosine, tGSH- total glutathione levels, GSSG- glutathione disulphide (oxidized form), GSH-glutathione (reduced form), SOD-superoxide dismutase, FEV_1- Forced Expiratory Volume in one second, FVC- Forced Vital capacity, FEV_1/FVC -Forced Expiratory Ratio.

was observed in all workers; the stratified at-risk (p=0.024) and mild (p=0.004) COPD cases also had significant DNA damage compared to controls while the mild COPD cases had more DNA damage (p=0.037) compared to those with moderate COPD. The lower damage in moderate COPD cases may be because of fewer individuals in that group and their differing genotypes/lifestyles/habits. Moreover the common oxidized bases become reactive intermediates under conditions of oxidative stress (Burrows et al. 2002) and the 8-OHdG moiety has the tendency on further oxidation to form hydantoin products (which have the same mutagenic potential and are subject to the action of the same repair enzymes as the parental 8-OHdG). Since the moderate COPD subjects are under greater oxidative stress than those with the mild COPD, therefore, after processing of 8-OHdG by oxidants, there are probably fewer intact 8-OHdG groups in the moderate than in the mild COPD. The marked inflammation in COPD can further worsen the oxidant/antioxidant balance and in this way may be promoting the disruption of genomic integrity (Boots et al. 2003).

Genetic damage has been observed in similar occupational environments: in road-tunnel construction workers, the micronuclei frequency in PBL was increased (Villarini et al. 2008), in calcite factory workers, higher micronuclei and nuclear abnormalities in buccal cells were observed (Diler and Ergene 2010), and in those involved in mining, stone-crushing, grinding and sandblasting, increased micronuclei, both in PBL and in nasal epithelial cells, were reported (Demircigil et al. 2010). Workers engaged in stone crushing, grinding, carrying chips, and extracting brick earths were reported to have increased genetic damage both in peripheral blood lymphocytes and the buccal mucosal cells (Halder and De 2012). Workers engaged in crushing and quarrying stones were also reported to have significant chromosomal damage in their urothelial cells (Kaur and Gandhi 2011a, 2011b) and an increase in chromosomal aberrations in the PBL had also been earlier documented (Sob-

ti and Bhardwaj 1991). The blood levels of 8-OHdG were significantly raised in lung cancer patients (Gackowski et al. 2003) while the cytokinesis block micronucleus assay in COPD disease showed increased micronuclei (MN) frequency (Maluf et al. 2007) though no increase in sister chromatid-exchanges (SCEs) or MN were observed in COPD cases (Casella et al. 2006). However, 8-OHdG levels were elevated in COPD cases (Caramori et al. 2001; Tzortzaki et al. 2012) with increase in DNA strand breaks (Ceylan et al. 2006) and damage index (da Silva et al. 2013b); oxidative damage in RNA and DNA in COPD cases has also been reported (Drost et al. 2005; MacNee 2005; Yoshida and Tuder 2007). The observations on DNA damage in the present study hence are similar to those in literature.

Oxidative Stress

All workers had significantly lowered total GSH ($p=0.046$) and SOD ($p=0.021$) levels implying the presence of oxidative stress (Table 2). As also observed for oxidative DNA damage, the at- risk and mild COPD cases, respectively had significantly decreased tGSH ($p=0.001$, $p=0.018$) and SOD ($p=0.046$, $p=0.024$) levels from controls. In the moderate COPD cases significantly ($p=0.038$) elevated tGSH levels in comparison to those at-risk for COPD were observed. The group comprised fewer individuals; they may have higher antioxidant levels and variant life-styles accounting for the comparatively lesser oxidative-stress vis-à-vis in mild cases.

In the literature also oxidative stress has been documented in COPD cases. Low erythrocyte glutathione peroxidase (GP_X) activity and higher serum malondialdehyde levels in severe COPD cases were reported (Kluchová et al. 2007) in comparison to values in moderate COPD cases, but there were no differences in the erythrocyte SOD and CAT activities between the patients differing for severity of COPD. Oxidative stress in COPD and in lung dysfunction was observed as decreased levels of glutathione, superoxide dismutase, catalase, antioxidants (ascorbic acid, vitamin E) and increased lipid peroxidation (Kluchová et al. 2007; Borm et al. 2004; Tug et al. 2005; da Silva et al. 2013a).

Disease pathogenesis involves cell damage induced by superoxide radical ($O_2^{\cdot-}$) and related oxygen species (Mak et al. 2007).The enzymes SOD, CAT and GP_X neutralize free radicals and prevent the transformation of the superoxide to hydroxyl radicals (Borm et al. 2004). SOD converts $O_2^{\cdot-}$ to hydrogen peroxide, while GP_X (using reduced GSH as hydrogen donor) and catalase transform it to water (Mak et al. 2007). Changes in activities of these enzymes or their absence can hence cause oxidative stress. In fact oxidative stress is among the pathological hallmarks in the development of COPD (Boots et al. 2003); this was also observed in COPD cases of present study as decreased SOD and GSH levels. The detrimental effects of oxidative stress include impairment of membrane functions, inactivation of membrane-bound receptors and enzymes, and increased tissue permeability (Kluchová et al. 2007). In addition to these, oxidative stress aggravates inflammatory processes in lungs and worsens the oxidant/antioxidant balance (Boots et al. 2003).

Oxidative DNA Damage and Oxidative Stress

A significant inverse association of enzymatic levels (tGSH: $r=-0.353$, $p=0.047$; SOD: $r=-0.721$, $p=0.000$) with oxidative DNA damage (8-OHdG levels) in COPD cases was apparent on correlation and multiple linear regression analyses (Table 6). This association also extended to levels of GSSG($r=-0.299$, $p=0.033$) and GSH($r=-0.362$, $p=0.009$) verifying that with decreased enzymatic levels, oxidative stress is not neutralized and manifests as the observed oxidative DNA damage.

Both oxidative DNA damage and oxidative stress have together also been earlier documented in literature which substantiates that oxidative stress in all probability is causing oxidative DNA damage. DNA damage and oxidative stress were markedly increased in COPD cases (Ceylan et al. 2006) while increased apoptosis because of excessive genetic damage from prolonged oxidative stress in lymphocytes from airways of COPD patients was also reported (Segura-Valdez et al. 2000; Majo et al. 2001; Hodge et al. 2003).

Genotyping

Since besides oxidative stress, the development and progression of COPD also involves multiple genes, gene-gene and gene-environment interactions (•idzik et al. 2008), genotyping of three allelic variants of the *GST* multigene

family (phase II enzymes) and one of *MnSOD* was carried out (Table 3). The reactive oxygen species generated by phase I enzymes get converted to inactive derivatives by phase II enzymes (*GST* multigene family). The *GSTT1* and *GSTM1* null genotypes show no enzyme activity and the *GSTP* Val allele has reduced enzyme activity (Hiragi et al. 2011). The *MnSOD* precursor protein with Ala signal peptide is essential for its efficient mitochondrial transport and it accounts for ~90 percent of the total SOD activity while the *Val* substitution causes reduced SOD activity (Mak et al. 2007).

The frequencies obtained of the variant genotypes were generally low, viz. *GSTT1*+/- (75.00%, 25.00%), M1+/- (56.25%, 43.75%) and P1++/+-/— (87.50%, 3.12%, 9.37%) and of *MnSOD* (Val/Val-53.12%; Ala/Val-31.25%;Ala/Ala-6.12%) in COPD cases. In literature, a range of frequencies of these genotypes exist for COPD cases from China (Chan-Yeung et al. 2007), Turkey (Çalikoglu et al. 2006) and in Caucasians (Siedlinski et al. 2009). Rather, the frequency of *GSTM1* null genotype is within the reported range of 40-50 percent in Caucasians (Moretti et al. 2007). On comparison of the genotypes in COPD cases and controls, no significant differences were forthcoming implying that these genotypes showed no association with disease occurrence and/or lung dysfunction. Combinational genotypes also failed to yield any genetic predisposition to COPD. This is at variance with some results in literature for lung function/COPD. The presence of microsomal epoxide hydroxylase (EPHX1) His113- His113 variant and *GSTM1* null genotype was a significant predictor for increased susceptibility to COPD in the Slovak population (Zidzik et al. 2008). The *GSTT1* null genotype (alone or in combination with *GSTM* null genotype) was associated with decline in FEV_1 in SAPALDIA cohort study comprising European caucasian ethnicity and Swiss nationality (Imboden et al. 2007). In central Tunisia, the *GSTM1* null/Val/Val *GSTP*1 combination genotype was associated with COPD (Lakhdar et al. 2011). However other reports match with those

Table 3: Distribution of genotypes and allele frequencies in the study group

Genetic Polymorphism	*COPD cases n (%)*	*Controls n (%)*	χ^2 *value*	*P value*
Genotypes				
GSTT1(†rs17856199)				
Present	24(75.00)	16(84.21)	0.170	0.67
Null	08(25.00)	03(15.78)		
GSTM1(†rs366631)				
Present	18(56.25)	13(68.42)	0.310	0.57
Null	14(43.75)	06(31.57)		
GSTP1(Ile/Val) (†rs1695)				
Ile/Ile	28(87.5)	15(78.94)	2.790	0.24
Ile/Val	01(3.12)	03(15.78)		
Val/Val	03(9.37)	01(5.26)		
MnSOD(Val/Ala)(†rs179972)				
Val/Val	17(53.12)	13(68.42)	1.150	0.56
Ala/Val	10(31.25)	04(21.05)		
Ala/Ala	05(16.12)	02(10.52)		
Alleles				
††*GSTT1*				
Present	32(50.00)	23(60.52)	0.680	0.41
Null	32(50.00)	15(39.47)		
††*GSTM1*				
Present	22(34.37)	17(44.73)	0.690	0.41
Null	42(65.62)	21(55.26)		
GSTP(Ile/Val)				
Ile	57(89.06)	33(86.80)	0.000	0.98
Val	07(10.93)	05(13.15)		
MnSOD(Val/Ala)				
Val	44(68.75)	30(78.94)	0.780	0.37
Ala	20(31.25)	08(21.05)		

Significant at p value <0.05, †SNP database Id(Gene cards). ††Hardy-Weinberg equation
GSTT1-glutathione S-transferase theta-1, *GSTM1*-glutathione S-transferase mu 1, *GSTP1*-glutathione S-transferase pi, *MnSOD*- manganese superoxide dismutase;
GSTT1 and *GSTM1*: present (wild-type homozygous or heterozygous) and null (homozygous gene deletion).

in the present study: in Koreans no association of *GST*1 and *GSTM1* was observed with COPD (Yim et al. 2000). The Ala variant of *MnSOD* in the Chinese exhibited increased risk for lung cancer but not for COPD (Wang et al. 2001), and in Hong Kong Chinese similarly, both *MnSOD* and catalase genes did not show predisposition for COPD (Mak et al. 2007).

Genetic Polymorphism and Oxidative DNA Damage

Significantly elevated 8-OHdG levels (Table 4) were obtained in COPD cases with the *GSTM1* null (p=0.000), *GST*P1 Val/Val (p=0.000) and *MnSOD* Val/Ala, Ala/Ala (p=0.000) genotypes.

Studies from literature have also revealed that the *GST* and *MnSOD* variants are associated with increased genetic damage. The *GSTM1* null genotype was associated with higher levels of 8-OHdG (Mirzaei et al. 2014).The *GST*P1 (Val/Val) genotype was associated with increased DNA tail moment (Liu et al. 2006) and the *MnSOD* Val/Ala or Ala/Ala genotypes with MPO G/G genotype had higher urinary 8-OHdG concentrations on PAH exposure (Park et al. 2006).

Genetic Polymorphism and Oxidative Stress

The t GSH levels were significantly (p=0.003) lower in COPD cases with *GST*P1 Val/Val genotype compared to those with the Ala/Ala genotype. Significantly lower SOD levels were also observed in COPD cases with *GSTT1* null (p=0.002) and *GSTM1* null (p=0.004) compared respectively with those having these alleles, As expected, the cases with *MnSOD* Val/Ala or the *MnSOD* Ala/Ala genotypes had significantly (p=0.000) lowered SOD levels compared to those with the *MnSOD* Val/Val genotype (Table 4).

In reports in literature, an association of increased oxidative stress with genetic polymorphism has been documented. Significant lower GP_x, GR, SOD, GSH and TAS levels were observed in COPD patients and their correlation was observed with the double null *GSTM1*/*GSTT1*, and *GSTM1*null/*GST*P1Val/Val combinational genotypes (Lakhdar et al. 2011).

Oxidative DNA Damage, Oxidative Stress and Genotype Interactions

In order to study the association of different genotypes with oxidative DNA damage, only the *GSTT*1/*GSTM1* combinations and those with sufficient study participants were analyzed (Table 4). COPD cases with *GSTT1*present /*GSTM1* null genotypes had significantly (p=0.000) elevated 8-OHdG levels compared to the *GSTT1*/M1 present genotypes. A significant increase in oxidative DNA damage was also observed in COPD cases with the *GSTT1* null /*GSTM1* null genotype compared to those with *GSTT1* null/*GSTM1* present genotype (p=0.054). Significantly lowered t GSH levels were observed in COPD cases with *GSTT1* null/*GSTM1* null (p=0.000) and *GSTT1* present /*GSTM1*null (p=0.021). For SOD, low levels were observed in COPD cases with *GST* (*GSTT1*present/*GSTM1*null (p=0.000), *GSTT1*null/*GSTM1*present (p=0.049) and *GSTT1*null/*GSTM1* null (p=0.000) combinational genotypes in comparison to those with the *GSTT1* present/*GSTM1*present combinational genotype.

Studies in literature also showcase such observations. The *MnSOD* Val/Ala had less DNA damage and lower lipid peroxidation (Miranda-Vilela et al. 2010) while the *MnSOD* Val/Val genotype was more frequent in COPD patients and showed lower expression of *MnSOD* mRNA (Pietras et al. 2010). Both 8-OHdG in DNA and 8-OHdGuanine in RNA were increased in lung diseases (Deslee et al. 2010).

Genetic Predisposition to COPD

To assess whether the genetic variants of *GST* and *MnSOD* genes could be predictors for pre-disposition to COPD (Table 5), the odds ratio was calculated (without adjustment for sex, age and SES since these variables were matched for cases and controls) taking the wild type genotypes as referents. None of the genotypes in the study group showed any significance or likelihood for developing COPD.

Reports in literature for COPD cases are lacking. However, children with asthma having the *GSTM1* null and Val/Val *GST*P1 genotypes had abnormal pulmonary functions (Gilliland et al. 2002) and the *GST*P1 Ile/Ile genotype showed association with chronic lung disease (Manar et al. 2004).

Predictive factors for Genetic Damage

Taking 8-OHdG as a dependent variable, the analysis of correlation and multiple linear regression revealed that genetic damage was signifi-

Table 4: Oxidative DNA damage and oxidative stress as functions of genetic polymorphisms in COPD cases and healthy subjects

Genetic polymorphism		*COPD cases*				*Controls*			
		n	*8-OHdG (Mean± S.E.M.)*	*tGSH†(Mean ± S.E.M.)*	*SOD (Mean± S.E.M.)*	*n*	*8-OHdG (Mean± S.E.M.)*	*tGSH †(Mean±S.E.M.)*	*SOD (Mean±S.E.M.)*
MnSOD	Val/Val	17	$0.27^{*}\pm 0.09^{a}$	221.63 ± 1.31	100.10 ± 0.5^{a}	13	0.04 ± 0.00^{a}	$226.74^{**}\pm 0.84^{a}$	101.39 ± 0.43^{a}
	Val/Ala	10	0.87 ± 0.15^{b}	217.45 ± 2.28	88.53 ± 2.45^{a}	04	0.30 ± 0.24^{b}	223.84 ± 2.35^{b}	$100.42^{**}\pm 0.80^{a}$
	Ala/Ala	5	0.85 ± 0.17^{b}	193.37 ± 20.27	87.23 ± 2.04^{b}	02	1.12 ± 0.009^{b}	219.76 ± 0.64	84.08 ± 4.60^{b}
GSTP1	Ile/Ile	28	$0.52^{**}\pm 0.09$	218.88 ± 1.21^{a}	94.74 ± 1.42	15	0.11 ± 0.07^{a}	$226.26^{***}\pm 0.95^{a}$	$100.59^{**}\pm 0.94^{a}$
	Ile/Val	1	0.02 ± 0.00^{a}	228.33 ± 0.00	102.34 ± 0.00	03	0.755 ± 0.33^{b}	221.00 ± 1.63^{b}	93.12 ± 6.82^{b}
	Val/Val	3	$1.06^{***}\pm 0.00^{b}$	183.99 ± 35.85^{b}	89.39 ± 5.64	01	0.078 ± 0.00	225.67 ± 0.00	99.68 ± 0.00
GSTT1	Present	24	$0.51^{*}\pm 0.10$	219.54 ± 1.20	95.28 ± 1.52^{a}	16	0.17 ± 0.08	$225.87^{***}\pm 0.96$	$100.43^{**}\pm 0.87^{a}$
	Null	8	0.69 ± 0.15	205.02 ± 13.50	92.06 ± 3.02^{b}	03	0.42 ± 0.35	222.28 ± 2.29	93.68 ± 7.16^{b}
GSTM1	Present	18	$0.24^{*}\pm 0.08^{a}$	221.97 ± 1.54	97.97 ± 1.38^{a}	13	0.03 ± 0.005^{a}	$227.16^{**}\pm 0.84^{a}$	$101.60^{*}\pm 0.43$
	Null	14	0.95 ± 0.10^{b}	208.12 ± 7.42	89.97 ± 2.06^{b}	06	0.59 ± 0.22^{b}	221.59 ± 1.10^{b}	94.51 ± 3.50
††Gene-Gene Combinations									
GSTT1(+)/GSTM1(+)		13	0.16 ± 0.08^{a}	223.63 ± 1.18^{a}	99.63 ± 1.04^{a}	11	0.03 ± 0.005^{a}	$227.60^{**}\pm 0.89^{a}$	101.75 ± 0.45^{a}
GST1(+)/GSTM1 (-)		10	0.91 ± 0.14^{b}	214.48 ± 1.04^{b}	89.21 ± 2.32^{b}	6	0.59 ± 0.22^{b}	$221.59^{***}\pm 1.10$	94.51 ± 3.50
GST1(-)/GSTM1 (+)		5	0.46 ± 0.18^{c1}	217.63 ± 4.37	93.65 ± 3.78^{b}	2	0.06 ± 0.00^{c1}	224.74 ± 2.29	100.79 ± 1.61
GST1(-)/GSTM1 (-)		3	1.06 ± 0.00^{c2}	183.99 ± 35.85^{b}	89.39 ± 5.64^{b}	1	$1.13^{*}\pm 0.00^{c2}$	219.12 ± 0.00^{b}	79.48 ± 0.00^{b}

***very highly significant ($p\leq 0.001$), **highly significant($p\leq 0.01$) , *significant ($p<0.05$, Student's t-test) compared to controls.
Values with different letters and number (gene-gene combination) are significant within group.
GSTT1-glutathione S-transferase theta-1, *GSTM1*-glutathione S-transferase mu 1, *GSTP1*-glutathione S-transferase pi, *MnSOD*- manganese superoxide dismutase; *GSTT1* and *GSTM1*: + (present;wild-type homozygous or heterozygous) and - (null;homozygous gene deletion).
8-OHdG-8- hydroxy-22 -deoxyguanosine, tGSH- total glutathione levels, SOD-superoxide dismutase.
†only tGSH significantly lower in COPD cases than the controls so analysed;
††*GST* (*T1*, *M1* and *P1*) and *MnSOD* combinations not analysed because of lesser number in the groups.

Table 5: Predictor assessment for COPD on the basis of genetic polymorphisms

Genetic Polymorphism		*COPD cases*	*Con-trols*	*Refe-rent*	*Odds Ratio (95% CI)*	*P value*
GSTT1	*Present*	24	16	1.00	0.56 (0.12-2.44)	0.440
	Null	8	3			
GSTM1	*Present*	18	13	1.00	0.59 (0.18-1.95)	0.390
	Null	14	6			
GSTP1(Ile/Val)	*Ile/Ile*	28	15	1.00	5.6 (0.53-58.63)	0.150
	Ile/Val	1	3			
	Val/Val	03	01		0.62 (0.05-6.51)	0.690
	Ile/Ile+Ile/Val	29	18	1.00		
	Val/Val	03	01		1.611 (0.29-8.86)	0.580
MnSOD(Val/Ala)	*Val/Val*	17	13	1.00	0.52 (0.13-2.05)	0.350
	Val/Ala	10	04			
	Ala / Ala	05	02		0.52 (0.08-3.13)	0.470
	Val/Val + Val/Ala	27	17	1.00	0.63 (0.11-3.65)	0.610
	Ala/Ala	05	02			
Gene-Gene Combinations						
GSTT1/GSTM1	Present / Present	13	11	1.00		
	Present / Null	11	04		0.429 (0.106-1.73)	0.230
	Null / Present	04	02		0.59 (0.09-3.86)	0.580
	Null / Null	03	01		0.39 (0.03-4.34)	0.440
GSTT1(+), *GSTM1*(+), *GSTP1* Ile/Ile, *MnSOD* Val/Val		11	10	1.00		
GSTM1 (-), *GSTT1*(-), *GSTP1*Val/Val, *MnSOD* Ala / Ala		01	-		-	
GSTT1(+), GSTM1 (-), GSTP1 Ile/Ile, *MnSOD* Val/Val		03	-		-	
GSTT1(+), GSTM1 (-), GSTP1 Ile/Ile, *MnSOD* Val/Ala		07	01		0.15(0.01-1.51)	0.100
GSTM1 (-), *GSTT1*(-), *GSTP1* Val/Val, *MnSOD* Val/Val		02	-		-	
GSTT1(+), *GSTM1*(+), *GSTP1 Ile/Ile*, *MnSOD* Ala / Ala		01	-		-	
GSTT1(+), *GSTM1 (-), GSTP1 Ile/Ile*, *MnSOD* Ala / Ala		01	-		-	
GSTT1(+), *GSTM1*(+), *GSTP1 Ile/Ile*, *MnSOD* Val/Ala		01	01		1(0.06-20.01)	0.948
GSTT1(-), GSTM1(+), GSTP1 Ile/Ile, *MnSOD* Val/Ala		01	-		-	
GSTT1(-), GSTM1(+), GSTP1 Val/Val, *MnSOD* Ala / Ala		02	-		-	
GSTT1(-), GSTM1(+), GSTP1 Ile/Ile, *MnSOD* Ala / Ala		01	-		-	
GSTT1(+), GSTM1 (-), GSTP1 Ile/Ile, *MnSOD* Ala / Ala		01	01		1(0.06-20.01)	0.948
GSTT1(-), GSTM1(+), GSTP1 Ile/Ile, MnSOD Val/Val		-	02		-	-
GSTT1(+), GSTM1 (-), GSTP1 Val/Val, *MnSOD* Val/Ala		-	01		-	-
GSTT1(+), GSTM1 (-), GSTP1 Ile/ Val, *MnSOD* Val/Val		-	01		-	-
GSTT1(+), GSTM1 (-), GSTP1 Ile/ Val, *MnSOD* Val/Ala		-	01		-	-
GSTM1 (-), GSTT1(-), GSTP1 Ile/ Val, *MnSOD* Ala / Ala		-	01		-	-
Allelic distribution						
†*GSTT1*						
Present		32	23	1.00	0.65(0.28-1.47)	0.300
Null		32	15			
†*GSTM1*						
Present		22	17	1.00	0.65(0.28-1.47)	0.290
Null		42	21			
GSTP1						
Ile		57	33	1.00	1.23(0.36-4.20)	0.730
Val		07	05			
MnSOD						
Val		44	30	1.00	0.58(0.22-1.50)	0.260
Ala		20	08			

$p<0.05$ is significant, CI- confidence interval. † Hardy-Weinberg equation
GSTT1-glutathione S-transferase theta-1, *GSTM1*-glutathione S-transferase mu 1, *GSTP1*-glutathione S-transferase pi, *MnSOD*- manganese superoxide dismutase; *GSTT1* and *GSTM1*: + (present; wild-type homozygous or heterozygous) and - (null; homozygous gene deletion).

cantly associated with the levels of tGSH and GSSG, GSH, SOD as well as the *GSTM1*, *GST*P1 and *MnSOD* genotypes (Table 6). Hence these can all be also considered as predictive factors for genetic damage in the studied population. Of these, the *GSTM1* and *MnSOD* genotypes as well as the SOD enzyme were highly significant (p=0.000) predictors.

Table 6: Predictive factors for genetic damage (8-OHdG level[a])

Variables	*COPD cases*				*Controls*				*Total study group*			
	Pearson Correlation	*Multiple linear regression*		*P value*	*Pearson Correlation*	*Multiple linear regression*		*P value*	*Pearson Correlation*	*Multiple linear regression*		*P value*
	r	*β*	*t*		*r*	*β*	*t*		*r*	*β*	*t*	
tGSH	-0.353	-0.353	-2.068	**0.047**	-0.681	-0.681	-3.839	**0.001**	-0.413	-0.413	-3.174	**0.003**
GSSG	-0.285	-0.285	-1.626	0.115	-0.185	-0.185	-0.776	0.448	-0.299	-0.299	-2.192	**0.033**
GSH	-0.311	-0.311	-1.793	0.083	-0.486	-0.486	-2.292	**0.035**	-0.362	-0.362	-2.719	**0.009**
Redox Index	0.015	0.015	0.083	0.935	0.062	0.062	0.256	0.801	0.037	0.037	0.260	0.796
SOD	-0.721	-0.721	-5.691	**0.000**	-0.801	-0.801	-5.517	**0.000**	-0.769	-0.769	-8.417	**0.000**
*GSTT1(-)*vs *GSTT1(+)*	0.157	0.157	0.8691	0.392	0.237	0.237	1.005	0.329	0.204	0.204	1.459	0.151
*GSTM1(-)*vs *GSTT1(+)*	0.703	0.703	5.407	**0.000**	0.667	0.667	3.687	**0.002**	0.684	0.684	6.570	**0.000**
GSTP(Ile/Ile and *Ile/Val* vs *Val/Val)*	0.187	0.187	1.042	0.306	0.499	0.499	2.374	**0.030**	0.234	0.234	1.688	0.098
MnSOD(Val / Val and *Val/Ala* vs *Ala/ Ala)*	0.593	0.593	4.030	**0.000**	0.650	0.650	3.522	**0.003**	0.616	0.616	5.472	**0.000**

[a]dependent variable is leukocyte DNA 8-OHdG level. values in bold are significant ($p<0.05$).
GSTT1-glutathione S-transferase theta-1, *GSTM1*-glutathione S-transferase mu 1, *GSTP1*-glutathione S-transferase pi,
MnSOD- manganese superoxide dismutase; 8-OHdG-8-hydroxy-22 -deoxyguanosine,
tGSH- total glutathione levels, GSSG- glutathione disulphide (oxidized form), GSH-glutathione (reduced form), SOD-superoxide dismutase.
GSTT1 and *GSTM1*: + (present; wild-type homozygous or heterozygous) and - (null; homozygous gene deletion).

Prognostic factors for DNA Damage– Calculation of Hazard Ratio

In Table 7, the significant prognostic factors for both oxidative stress and genetic damage are shown. Of these, FEV_1 among the lung function values (HR16.48, 95% CI 238.32-100.9), SOD levels (HR 1.351, 95%CI 1.07-1.69) among oxidative stress parameters, the *GSTM1* genotype (HR 0.091, 95% CI 0.001-0.374%) and alcohol drinking habit (HR 0.178, 95%CI 0.031-1.033) were revealed to be significant ($p \leq 0.05$) prognostic factors. As oxidative stress and oxidative DNA damage are correlated ($p \leq 0.05$), the observed significantly($p \leq 0.05$) raised 8-OHdG level in COPD cases has probably occurred because of the cellular oxidative stress arising from the decreased GSH, GSSG, SOD levels. In the literature also, greater levels of oxidative stress were observed to show greatest oxidative damage to leukocytic DNA (Liu et al. 2009). The 8-OHdG results from the oxidation of DNA (on interaction of hydroxyl ions with guanine) and becomes pro-mutagenic (Gackowski et al. 2003; Kasai 1997). The oxidative mechanisms produce reactive oxygen species (ROS) and exacerbate the generation of molecular oxygen and the superoxide and hydroxyl radicals. The 8-OHdG resulting from the oxidation of DNA as hydroxyl radicals interact with guanine makes it pre-mutagenic (Valavanidis et al. 2009). This oxidized DNA product has been documented to further promote/ exacerbate oxidative stress and result in ageing, malignancy, tumors and neurodegenerative disease (Rahman 2007; Valko et al. 2007). Both alcohol drinking and FEV_1 depicted an increased hazard risk for oxidative stress and oxidative DNA damage. Alcohol intake has been observed to increase chromosomal damage (Kalaiselvi et al. 2002) and its association with oxidative stress and oxidative DNA damage in the present study, matches reports in literature: alcohol consumption elevated genetic damage (Fenech et al. 2007), oxidative stress (Albano 2006) and was associated with decline in lung function (Siu et al. 2010). Among the genotypes, the *GSTM1* variant in the ethnic group of the present study with very highly significant (p=0.001) HR (0.091, 95%CI

Table 7: Prognostic factors for oxidative stress and genetic damage and prognostic score of COPD cases

Prognostic factors	*Hazards ratio (95%CI)*		*β*	*P value*	*Prognostic score of COPD cases Mean±S.E.M.(range)*
FEV_1	16.48	(1.54 - 175.54)	2.802	**0.020**	42.46±0.95(31.94 - 49.81)
SOD1.351		(1.07 - 1.69)	0.301	**0.009**	
GSTM1	0.091	(0.001- 0.374)	3.973	**0.009**	
Alcohol drinking	0.178	(0.031- 1.033)	1.726	**0.054**	
Age0.924		(0.793- 1.076)	-0.079	0.309	-
BMI	0.850	(0.411- 1.757)	-0.162	0.661	
BI	1.004	(0.977- 1.032)	0.004	0.773	
Smoking	0.174	(0.014- 2.164)	-1.748	0.174	
Tobacco	1.604	(0.126- 20.42)	0.472	0.716	
FVC	1.240	(0.344- 4.476)	0.215	0.742	
FEV_1	0.346	(0.00 - 217.519)	-4.994	**0.000**	
tGSH	0.584	(0.709- 1.839)	0.133	0.584	
GSSG	0.498	(0.012- 8.593)	-1.136	0.321	
GSTT1	0.298	(0.031- 2.911)	-1.209	0.298	
GSTP1(Ile/Val)	1.239	(0.154- 9.965)	0.214	0.841	
MnSOD(Val/Ala)	2.897	(0.075- 112.274)	1.064	0.569	

COPD status	*Prognostic Score Mean± S.E.M.Student's t-test*		*P-value*
At-risk	45.74±0.76	At-risk versus Mild	**0.015**
Mild	41.25±1.81	At-risk versus Moderate	**0.000**
Moderate	35.88±0.39	Mild versus Moderate	**0.042**

Values in bold are significant (P<0.05; multiple Cox proportional hazards model, Student's t-test),
CI- confidence interval, BMI-body mass index, FEV_1- Forced Expiratory Volume in one second,
BMI-body mass index, BI-brinkman index, FVC-forced vital capacity, tGSH- total glutathione levels,
GSSH- glutathione disulphide (oxidized form), SOD-superoxide dismutase,
GSTM1-glutathione S-transferase mu 1.

0.001-0.374) is also a strong prognostic factor for DNA damage following SOD (HR 1.351, 95% CI 1.07-1.69) and FEV_1 (HR 16.48, 95%CI 1.54-175.54). The *GSTM1* null genotype has also been associated with increased genetic damage viz. increased DNA tail moment (Singh et al. 2012) and 8-OHdG levels (Kim et al., 2003). The former is the product of length of tail of DNA and the percentage of DNA in the tail. If damage/ breaks in DNA have occurred and the cells are subjected to electrophoresis, fragmented DNA will move out of the nucleoid causing tail formation (comet), which is observable using the single cell gel electrophoresis assay.

Prognostic Score/Index for Oxidative DNA Damage

A prognostic score (Table 7) as a liability index for genetic damage was developed as this was the main purpose of the present study. Such an index can by extension be used for cancer risk susceptibility since genetic damage (especially 8-OHdG) is an earlier indicator of cancer. For the formulation of the index it was required that the independent COPD prognostic risk factors deterministic for genetic damage be considered based on the model (survival analysis) proposed for Mantle Cell International Prognostic Index (MIPI) (Hoster et al. 2008).The significant prognostic risk factors obtained (Table 7) in the present study were considered for developing the prognostic index using the Cox proportional hazard model**.** The median prognostic score was 42.56 (range 31.94-49.81) with a mean score of 42.46±0.95. Considering that 10% of the score values were below 41.94 and 90% below 43.41, potential cut-off points were set between 41.93 and 43.43 in steps of 0.05 were assessed for increased likelihood for genetic damage. On this basis, a high risk group of 17 COPD cases (53.12%, score ≥42.60) was identified. There were 11 cases (34.37%) at intermediate risk (37.07≤score<42.60) and the remaining four COPD cases (12.5%, score<37.07) belonging to the low risk group for genetic damage.

Stone-crushing Sites and Genetic Damage

The settled dust on the top-layer of the soil from stone-crushing units was collected and outsourced for analysis (Atomic Absorption Spectrophotometer and X-ray diffraction).Soil samples from all units contained lead, cadmium, copper, zinc, chromium and silica (personal communication). There was no statistically significant differences for oxidative DNA damage and oxidative stress in workers stratified for different stone-crushing units (Table 8). The concentration of various metals also varied only somewhat because of matching geochemical profile in these parts of Punjab indicating an almost similar kind of probable contributors to oxidative stress and DNA damage in the studied group.

As documented in literature, the heavy metals- sulphydryl group interactions in the enzymes result in structural enzyme deficiency /inhibition / disruption contributing to oxidative stress

Table 8: Genetic damage and oxidative stress in COPD cases as stratified for different stone-crushing units

Variables		*COPD cases*				
		Sarna (n=15)	*Gurdaspur (n=5)*	*Pathankot I (n=7)*	*Pathankot II (n=5)*	*Controls*
Oxidative DNA Damage	8-OHdG (Mean± S.E.M.)	0.55^{*}±0.13[a]	0.43±0.19	0.71^{**}±0.21[a]	0.4728±0.25	0.214±0.09[b]
Oxidative Stress Parameters	tGSH (Mean ± S.E.M.)	213.38±7.36	216.25±2.87[a]	220.14±2.84[a]	217.23±2.88[a]	$225.40^{***/*/**}$±0.89[b]
Parameters	SOD (Mean ±S.E.M.)	93.978±2.04[a]	91.730±3.72[a]	94.820±3.25	98.240±2.68	99.364^{*}±1.33[b]

***very highly significant ($p \leq 0.001$), **highly significant($p \leq 0.01$), *significant ($p<0.05$, Student's t-test) compared to controls. Values with different letters are significant from controls.
8-OHdG-8-hydroxy-22 -deoxyguanosine, tGSH- total glutathione levels, SOD-superoxide dismutase. Values with different letters are significant ($p<0.05$; Student's t-test)

(Hall 2002) and these, via free radicals can alter the structure of proteins, lipids and nucleic acids (Calderón et al. 2003) forming lesions, strand breaks, and base-modifications and so causing metal-induced carcinogenesis (Leonard et al. 2004). Heavy metal genotoxicity has also been documented: lead was genotoxic in peripheral blood lymphocytes and urine (Hengstler et al. 2003), cadmium in blood (Valverda et al. 2001), chromium in body fluids (Eastmond et al. 2008), cadmium and lead were genotoxic in bronchial epithelial cells (Glahn et al. 2008) and cadmium in urine was associated with 8-OHdG levels in non- smokers (Ketelslegers et al. 2008). Silica dust is also a proven genotoxicant (Basaran et al. 2003; Kim et al. 2010) and is carcinogenic (IARC 1997). The dust-particles containing silica and particulate matter (PM) from stone-crushing activities induce oxidative stress and lung damage (Villarini et al. 2008; Demircigil et al. 2010); silica dust, talc and particulate matter are both genotoxic and carcinogenic (Fatima et al. 2001; Albrecht et al. 2009). The dust and continued presence of these metals across the stone-crushing units hence makes them potential contributors towards oxidative stress and DNA damage observed in the workers.

The significantly increased DNA damage in the workers is a cause for concern in the light of associated potential risk for cancer documented in literature. Cancer etiology is linked to consequences of DNA modifications/DNA damage (apurinic/ apyrimididinic sites, oxidized purines or pyrimidines and strand breaks) and replication errors in somatic cells that give rise to mutations and subsequently cause promotion and development of the malignant tumour (Valavanidis et al. 2009). Unnrepaired DNA lesions in the cell cycle manifest as aneuploidy, chromosomal rearrangement and breakage-fusion-bridge cycles (Fenech 2002) and can trigger oncogenesis/DNA amplification. The unrepaired DNA lesions can also further promote oxidative stress and cause ageing, malignancy, tumors and neurodegenerative diseases(Valko et al. 2007).Diminished DNA repair capacity (from damaged DNA) can in turn cause transcription arrest, replication errors and genomic instability and initiate carcinogenesis (Jackson and Loeb 2001).

The observations of the present study corroborate those in the literature. The inhalation of air-borne dust at stone-crushing /quarrying and other dust-emitting occupations has been reported to cause ill-health such as respiratory distress (cough, phlegm, allergic disorders) and skin infections (Golbabaei et al. 2004; Ugbogu et al. 2009), as well as lung injury and inflammation(Vallyathan et al. 1995), tuberculosis (Tiwari et al. 2011), COPD (Jhoncy et al. 2011, Iftikhar et al. 2009), chronic renal and autoimmune diseases and even lung cancer (Gottesfeld et al. 2008).

Of the various mechanisms proposed in the pathogenesis of lung cancer and COPD, the shared pathways include oxidative stress, altered DNA replication, inflammation, angiogenesis and cellular proliferation/anti-apoptosis and epigenetic modifications including DNA methylation and histone modifications (Yang et al. 2011). From among these, the results of the present study have shown oxidative stress, DNA damage and genetic susceptibility in COPD cases. In the literature, there are not many reports on genetic damage assessment from exposure at this occupation (stone-crushing). The few studies have reported higher frequency of chromosomal aberrations and sister-chromatid-exchanges (Sobti and Bhardwaj 1991) and increased micronuclei (Kaur and Gandhi 2011a, 2011b) in stone crusher-unit workers. Some reports on elevated micronuclei frequency and increased comet tail length in PBL have also been presented, but in building-construction workers and in those with occupational exposure to silica dust (Demircigil et al. 2010; Sellappa et al. 2010; Halder and De 2012). The presence of oxidative stress from stone-crushing associated activities (as in the present study for tGSH and SOD) has also been documented (Tiwari et al. 2004). The *GSTM1* null genotype was also ascertained as a strong predictor for COPD in the present study as was also observed among the Slovaks (•idzik et al. 2008) and in Tunisians (Lakhdar et al. 2011); the *GSTM1* locus displayed loss of heterozygosity with a 15kb homozygous deletion (*GSTM1* null allele) in lung and bladder cancers (Ford et al. 2000; Lee et al. 2010). Genetic predisposition and/or a combination of susceptible genes and exposures including environmental factors (gene-environment) are known to contribute to cancer development and evidences suggest that COPD or impaired lung function is associated with lung cancer (Kiri et al. 2010). In the present study, *GSTM1*, FEV1, SOD, and alcohol drinking were significant prognostic factors for genetic damage. In the literature,

the *GSTM1* genotype has shown association with lung cancer (Carlston et al. 2008; Young et al. 2011b) and with FEV_1 decline (Imboden et al. 2007). As the 8-OHdG is pro-mutagenic (Gackowski et al. 2003; Kasai 1997) and is present in considerable amounts in malignancy and tumors (Rahman 2007; Valko et al. 2007), its increased level in the COPD cases of the present study make it a strong contender for initiating carcinogenesis. Furthermore, increased oxidative stress (SOD, t GSH, antioxidant depletion, etc.) as also observed in the present study, has been linked to cancer (Dincer et al. 2007; Abdalla 2011).

CONCLUSION

In conclusion, this is the first study to provide insights and exposure-effect relationship in workers exposed from occupational contaminants at stone-crushing sites. Despite the small study group, an effort nonetheless has been made to quantify at least, part of the inter-individual differences in relation to occupational exposure (thus enabling the identification of more susceptible groups for COPD 'missing cases'), genetic damage and eventually, future cancer-risk.

RECOMMENDATIONS

Similar studies with a greater number of samples can further elaborate on this. The knowledge about and targeting of common pathogenic mechanisms for lung cancer and COPD can provide potential diagnostic and therapeutic applications for patients with lung diseases as well as for those at-risk and also for establishing an effective surveillance. Also the investigations for changes at the genome level (as in the present study) are crucial in view of the possible implication(s) in progression from genetic damage, mutagenesis to neoplasia. The developing of prognostic scores and a prognostic index (based on survival analysis) for genetic damage (8-OHdG) risk and progression is a novel concept, albeit requiring validation in larger sample groups. Such an index has potential for wider ramifications for a host of other genetic damage end-points and can find applications, by extension for cancer-risk since all neoplasia have a genetic etiology.

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REFERENCES

Abdalla MY 2011. Glutathione as potential target for cancer therapy: More or less is good? (Mini-Review). *Jordan J Biol Sci,* 4: 119-124.

Albano E 2006. Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc,* 65: 278-290.

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker D, Tice R, Waters MD, Aitio A 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutat Res,* 463: 111–172.

Albrecht C, Knaapen AM, Demircigil GC, Coskun E, van Schooten FJ, Borm PJA, Schins RPF2009. Genomic instability in quartz test exposed rat lungs: Is inflammation responsible. *J Phys Conf Ser,* 151:1-8.

Aldrich MC, Munro HM, Mumma M, Grogan EL, Massion PP, Blackwell TS, Blot WJ 2015. Chronic Obstructive Pulmonary Disease and subsequent overall and lung cancer mortality in low-income adults. *PLoS ONE,* 10(3): e0121805. doi:10.1371/journal. pone. 0121805.

Barreiro E 2008. Chronic Obstructive Pulmonary Disease and lung cancer. *Arch Bronconeumol,* 44: 399-401.

Basaran N, Shubair M, Ündeger Ü, Kars A 2003. Monitoring of DNA damage in foundry and pottery workers exposed to silica by the alkaline comet assay. *Am J Ind Med,* 43: 602–610.

Bemt LV, Smeele IJM, Kolkman M, Grol R, Weel CV, Schermer TRJ 2010. Low body mass index, airflow obstruction, and dyspnea in a primary care COPD patient population. *Prim Care Respir J,* 19: 118-123.

Bernstein C 2012. DNA Damage and Cancer. Science Topics. From <http://www.scitopics.com/DNA_Damage_and_Cancer.html.> (Retrieved on 18 April 2012).

Boots AW, Haenen GR, Bast A 2003. Oxidant metabolism in Chronic Obstructive Pulmonary Disease. *Eur Respir J,* 46: 14-27.

Borm PJA, Schins RPF, Albrecht C 2004. Inhaled particles and lung cancer, Part B: Paradigms and risk assessment. *Int J Cancer,* 110: 3-14.

Briggs A, Spencer M, Wang H, Mannino D, Sin DD 2008. Development and validation of a prognostic index for health outcomes in Chronic Obstructive Pulmonary Disease. *Arch Intern Med,* 168: 71-79.

Burrows CJ, Muller JG, Kornyushyna O, Luo W, Duarte V, Leipold MD, David SS 2002. Structure and potential mutagenicity of new hydantoin prod-

ucts from guanosine and 8-oxo-7, 8-dihydroguanine oxidation by transition metals. *Environ Health Perspect*, 110: 713-717.

Cai Q, Shu X, Wen W, Cheng J, Qi D, Yu-Tang G, Zheng W 2004. Genetic polymorphism -in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: Results from the Shanghai breast cancer study. *Breast Cancer Res*, 6: R647-R655.

Calderón J, Ortiz-Pérez D, Yáñez L, Díaz-Barriga F 2003. Human exposure to metals: Pathways of exposure, biomarkers of effect, and host Factors. *Ecotox Environ Safe*, 56: 93-103.

Çalikoglu M, Tamer L, Aras NA, Karakas S, Ercan B 2006. The association between polymorphic genotypes of Glutothione S-Transferases and COPD in the Turkish population. *Biochem Genet*, 44: 307-319.

Caramori G, Casolari P, Cavallesco GN , Sarah GS, Adcock I, Papi A 2011. Mechanisms involved in lung cancer development in COPD. *Int J Biochem Cell Biol*, 43: 1030–1044.

Carlston C, Sagoo GS, Frodsham AJ, Burke W, Higgins JP 2008. Glutathione S Transferase M1 (*GSTM1*) polymorphism and lung cancer: A literature-based systematic huge review and meta-analysis. *Am J Epidemiol*, 167: 759-774.

Casella M, Miniati M, Monti S, Minichilli F, Bianchi F, Simi S 2006. No evidence of chromosome damage in Chronic Obstructive Pulmonary Disease (COPD). *Mutagenesis*, 21: 167-171.

Cazzola M, Hanania NA, MacNee W, Rüdell K, Hackford C, Tamimi N 2015. A review of the most common patient-reported outcomes in COPD – revisiting current knowledge and estimating future challenges. *International Journal of Chronic Obstructive Pulmonary Disease*, 10: 725-738.

Central Pollution Control Board 2009. *Comprehensive Industry Document Series: COINDS/78/2007-08*.

Ceylan E, Kocyigit A, Gencer M, Aksoy N, Selek S 2006. Increased DNA damage in patients with Chronic Obstructive Pulmonary disease who had once smoked or been exposed to biomass. *Respir Med*, 100: 1270-1276.

Chan-Yeung M, Ho SP, Cheung AHK, So LKY, Wong PC, Chan KK, Chan WM, Ip MSM, Mak JCW 2007. Polymorphisms of Glutathione-S-Transferase genes and functional activity in smokers with or without COPD. *Int J Tuberc Lung Dis*, 11: 508-514.

Chattopadhyay BP, Gangopadhyay PK, Bandopadhyay TS, Alam J 2006.Comparison of pulmonary function test abnormalities between stone crushing dust exposed and non-exposed agricultural workers. *Environ Health Prev Med*, 11: 191-198.

Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA1992. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G-T And A-C substitutions. *J Biol Chem*, 267: 166-172.

Crighton E J, Ragetlie R, Luo J, To T, Gershon A 2015. A spatial analysis of COPD prevalence, incidence, mortality and health service use in Ontario. *Health Reports*, 26: 10-18.

da Silva ALG, da Rosac HT, Charlier CF, Salvador M, Moura DJ, de Moura Valim AR, Guecheva TN, Henriques JAP 2013a. DNA damage and oxidative stress in patients with Chronic Obstructive Pulmonary Disease. *The Open Biomarkers Journal*, 6: 1-8.

da Silva ALG, da Rosa HT, Karnopp TE, Charlier CF, Ellwanger JH, Moura D J, Possuelo LG, de Moura AR, Guecheva TN, Henriques JAP 2013b. Evaluation of DNA damage in COPD patients and its correlation with polymorphisms in repair genes. *BMC Medical Genetics*, 14: 1-8.

Demircigil GC, Coskun E, Vidinli N, Erbay Y, Yilmaz M, Cimrin A, Schins RP, Borm PJ, Burgaz S 2010. Increased micronucleus frequencies in surrogate and targets cells from workers exposed to crystalline silica-containing dust. *Mutagenesis*, 25: 163-169.

Deslee G, Adair-Kirk TL, Betsuyaku T, Woods JC , Moore CH, Gierada DS, Conradi SH, Atkinson JJ , Toennies HM, Battaile JT, Kobayashi DK, Patterson GA, Holtzman MJ, Pierce RA 2010. Cigarette smoke induces nucleic acid oxidation in lung fibroblasts. *Am J Respir Cell Mol Biol*, 43: 576-584.

de Jong K, Vonk JM, Timens W, Bossé Y, Sin DD, Hao K, Kromhout H, Vermeulen R, Postma DS, Boezen HM 2015. Genome-wide interaction study of gene-by-occupational exposure and effects on FEV1 levels. *Journal of Allergy and Clinical Immunology*, doi:10.1016/j.jaci.2015.03.042.

Diler SB, Ergene S 2010. Nuclear anomalies in the buccal cells of calcite factory workers. *Genet Mol Biol*, 33: 374-378.

Dincer Y, Himmetoglu S, Akcay T, Ersoy EY, Gunes KN, Tortum O 2007. Prognostic significances of oxidative DNA damage evaluated by 8-Hydroxy-Deoxyguanosine and antioxidant enzymes in patients undergoing resection of gastric and colon carcinoma. *Neoplasma*, 54: 131-136.

Draid M M, Ben-Elhaj K M, Ali A M, Schmid K K, Gibbs S G 2015. Lung function impact from working in the pre-revolution Libyan quarry industry. *Int J Environ Res Public Health*, 12: 5006-5012.

Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti A, MacNee W 2005. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax, 60: 293-300.

Eastmond D, MacGregor J, Slesinski R 2008. Trivalent chromium: Assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. *Crit Rev Toxicol*, 38: 173-190.

Eisner MD, Blanc PD, Omachi TA, Yelin EH, Sidney S, Katz PP, Ackerson LM, Sanchez G, Tolstykh I, Iribarren C 2011. Socio-economic status, race and COPD health outcomes. *J Epidemiol Community Health*, 65: 26-34.

El-Zein RA, Young RP, Hopkins RJ, Etzel CJ 2012. Genetic predisposition to Chronic Obstructive Pulmonary Disease and/or lung cancer: Important considerations when evaluating risk. *Cancer Prev Res*, 5: 522-227.

Fatima SK, Prabhavathi PA, Padmavathi P, Reddy PP 2001. Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mutat Res*, 490:179-186.

Fenech M, Bolognesi C, Kirsch-Volders MS, Bonassi, Zeiger E, Knasmueller S, Holland N 2007. Harmonisation of the micronucleus assay in human buccal

cells—A Human Micronucleus (HUMN) Project (Www.Humn.Org) initiative commencing in 2007. *Mutagenesis*, 22: 3-4.

Fenech M, Holland N, Zeiger E, Chang WP, Burgaz S, Thomas P , Bolognesi C, Knasmueller S, Kirsch-Volders M, Bonassi S 2011.The HUMN and Humn$_x$ international collaboration project on human micronucleus assay in lymphocytes and buccal cells-past, present and future. *Mutagenesis*, 26: 239-245.

Fenech M 2002.Chromosomal biomarkers of genomic instability relevant to cancer. *Drug Discov,* 22: 1128-1137.

Ford JG, Li Y, O'Sullivan MM, Demopoulos R, Garte S, Taioli E, Brandt-Rauf PW 2000.Glutathione *S*-Transferase M1 polymorphism and lung cancer risk in African-Americans. *Carcinogenesis*, 2: 1971-1975.

Forlenza MJ, Miller GE 2006.Increased serum levels of 8-Hydroxy-2'-Deoxyguanosine in clinical depression. *Psychosom Med*, 68: 1-7.

Fortini P, Pascucci B, Parlanti E, D'Errico M, Simonelli V, Dogliotti E 2003. 8-Oxogaunine DNA Damage: At the crossroad of alternative repair pathways. *Mutat Res*, 531: 127-139.

Gackowski D, Speina E, Zielinska M, Kowalewski J, Rozalski R, Siomek A, Paciorek T, Tudek B, Olinski R 2003. Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. *Cancer Res*, 63: 4899–4902.

Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL 2005. Systemic reduction in Glutathione levels occurs in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*, 46: 877-883.

Ghotkar VB, Maldhure BR, Zodpey SP 1995. Involvement of lung and lung function tests in stone quarry workers. *Ind J Tub*, 42: 155-160.

Gilliland FD, Gauderman WJ, Vora H, Rappaport E, Dubeau L 2002. Effects of Glutathione-S-Transferase M1, T1 and P1 on childhood lung function growth. *Am J Respir Crit Care Med*, 166: 5710-5716.

Glahn F, Schmidt-Heck W, Zellmer S, Guthke R, Jan W, Golka K, Hergenröder R, Degen GH, Lehmann T, Hermes M, Schormann W, Brulport M, Bauer A, Bedawy E, Gebhardt R, Hengstler JG, Foth H 2008. Cadmium, cobalt and lead cause stress response, cell cycle deregulation and increased steroid as well as xenobiotic metabolism in primary normal human bronchial epithelial cells which is coordinated by at least nine transcription factors. *Arch Toxicol*, 82: 513-524.

GOLD (Global Initiative for Chronic Obstructive Lung Disease) 2003. Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease. NHLBI/WHO report.NIH, NHLBI publication Number 2701, Updated 2003. From <http://www.goldcopd.org/guidelineitem.asp.>

Golbabaei F, Barghi M, Sakhaei M 2004. Evaluation of workers' exposure to total, respirable and silica dust and the related health symptoms in Senjedak Stone Quarry. *Iran Ind Health*, 42: 29-33.

Gottesfeld P, Balakrishanan K, Rineheart R 2008.Reduction of respirable silica following the introduction of water spray applications in Indian stone crusher mills. *Int J Occup Environ Health*, 14: 94–103.

Hall JL 2002. Cellular mechanism for heavy metal detoxification and tolerance. *J Exp Bot*, 53: 1-11.

Halder A, De M 2012. Increase in DNA damage in lymphocytes and micronucleus frequency in buccal cells in silica-exposed workers. *Indian J Occup Environ Med*, 16: 34–37.

Hemminki K, Bermejo JL, Försti A 2006. The balance between heritable and environmental aetiology of human disease. *Nat Rev Genet*, 7: 958-965.

Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte W 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis*, 24: 3-73.

Hiragi CD, Miranada- Vilela AL, Rocha DMS, de Oliveira SF, Hatagima A, Klautau-Guimäes MD 2011. Superoxide dismutase, catalase, glutathione peroxidase and gluthatione S-transferases M1 and T1 gene polymorphisms in three Brazilian population groups. *Genet Mol Biol*, 34: 11–18.

Hillas G, Perlikos F, Tsiligianni I, Tzanakis N 2015. Managing comorbidities in COPD. *International Journal of Chronic Obstructive Pulmonary Disease*, 10: 95–109.

Hodge SJ, Hodge GL, Reynolds PN, Scicchitano R, Holmes M 2003. Increased production of TGF-Beta and apoptosis of T lymphocytes isolated from peripheral blood in COPD. *Am J Physiol Lung Cell Mol Physiol*, 285: L492–L499.

Hoster E, Dreyling M, Klapper W, Gissselbrecht C, Hoof A, Hanneke C, Kluin-Nelemans, Pfreundschuh M, Reiser M, Metzner B, Einsele H, Peter N, Jung W, Wormann B, Ludwig W, Duhrsen U, Eimermacher H, Wandt H, Hasford J, Hiddemann W, Unterhalt M 2008. A New Prognostic Index (MIPI) for patients with advanced-stage mantle cell lymphoma. *Blood*, 111: 558-65.

Iftikhar B, Khan MH, Hussain H, Iqbal M, Jadoon GS 2009. Relationship between silica dust exposure and Chronic Obstructive Pulmonary Disease in workers of dust generating industries of district Peshawar. *Gomal J Med Sci*, 7: 46-50.

Imboden M, Downs SH, Senn O, Matyas G, Brändli O, Russi EW, Schindler C, Ackermann-Liebrich U, Berger W, Probst-Hensch NM, SAPALDIA Team 2007. Glutathione S-Transferase genotypes modify lung function decline in the general population: SAPALDIA cohort study. *Respir Res*, 8:1-17.

IARC (International Agency for Research on Cancer) 1997. *Summaries and Evaluations*, 68: 41. Lyon, France.

Ito K, Barnes PJ 2009. COPD as a disease of accelerated lung aging. *Chest*, 135: 173-180.

Jackson AL, Loeb LA 2001. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mutat Res*, 477: 7-21.

Jhoncy SS, Ajay KT, Dhanyakumar G, Prabhu RN, Samuel V 2011. Effect of occupational exposure to dust on pulmonary function in workers associated with building demolition. *Biomed Res*, 22: 241-247.

Jindal SK 2006. Emergence of Chronic Obstructive Pulmonary Disease as an epidemic in India. *Indian J Med Res*, 124: 619-630.

Kalaiselvi K, Rajaguru, Palanivel M, Usharani MV, Ramu G 2002. Chromosomal aberration, micronucleus and comet assays on peripheral blood lympho-

cytes of leprosy patients undergoing multidrug treatment. *Mutagenesis,* 17: 309-312.
Kasai H 1997. Analysis of a form of oxidative DNA damage, 8-Hydroxy- 2_Deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res,* 387: 147–163.
Kaur G, Gandhi G 2011a. Preliminary Study for Cytogenetic Investigations in Workers at Stone-crushing Units. *Proceedings National Seminar on Management of Industrial Pollution,* 113-121.
Kaur G, Gandhi G 2011b. Genomic instability in workers with occupational exposure from stone- crushing activities. *Ind J Phys Anthrop and Hum Genet,* 30: 211-221.
Ketelslegers HB, Gottschalk RWH, Koppen G, Schoeters G, Baeyens WF, Larebeke NAV, Van Delft JHM, Kleinjans JCS 2008. Multiplex genotyping as a biomarker for susceptibility to carcinogenic exposure in the FLEHS biomonitoring study. *Cancer Epidemiol Biomarkers Prev,* 17: 1902-1912.
Kim YD, Lee CH, Nan, Kang JW, Kim H 2003. Effects of genetic polymorphism in metabolic enzymes on the relationship between 8-Hydroxydeoxyguanosine levels in human leukocytes and urinary 1-Hydroxypyrene and 2-Naphthol concentrations. *J Occu Health,* 45: 160-167.
Kim Y, Yu M, Park H, Yang S 2010. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by silica nanomaterials in human neuronal cell line. *Molecular and Cellular Toxicology,* 6: 336-343.
Kiri VA, Sorianob JB, Visickc G, Fabbri LM 2010. Recent trends in lung cancer and its association with COPD: An analysis using the UK GP research database. *Prim Care Respir,* 19: 57-61.
Kluchová Z, Petrášová D, Joppa P, Dorková Z, Tkáèová R 2007. The association between oxidative stress and obstructive lung impairment in patients with COPD. *Physiol Res,* 56: 51-56.
Kojima S, Sakakibara H, Motani S, Hirose K , Mizuno F, Ito M, Hashimoto S 2005. Effects of smoking and age on Chronic Obstructive Pulmonary Disease in Japan. *J Epidemiol,* 15: 113-117.
Kono J, Truscott TG, Martwell K 1978. Superoxide Dismutase was estimated in human being. *Am Heart J,* 22: 601-610.
Koshiol J, Rotunno M, Consonni D, Pesatori AC, Matteis SD, Goldstein AM, Chaturvedi AK, Wacholder S, Landi MT, Lubin JH, Caparaso NE 2009. Chronic Obstructive Pulmonary Disease and altered risk of lung cancer in a population- based case- control study. *PLoS ONE,* 4: 1-7.
Koster A, Bosma H, Kempen GIJM, Lenthe FJV, Eijk JTM, Mackenbach JP 2004. Socio-economic inequalities in mobility decline in Chronic Disease Groups (Asthma/COPD, Heart Disease, Diabetes Mellitus, Low Back Pain): Only a minor role for disease severity and comorbidity. *J Epidemiol Community Health,* 58: 862-869.
Kumar N, Shekhar C, Kumar P, Kundu AS 2007. Kuppuswamy's Socio-Economic Status Scale-updating for 2007. *Indian J Pediatr,* 74: 1131-1132.
Kurmi OP, Li L, Wang J, Millwood IY, Chen J, Collins R, Guo Y, Bian Z, Li J, Chen B, Xie K, Jia W, Gao Y, Peto R, Chen Z 2015. COPD and its association with smoking in the Mainland China: A cross-sectional analysis of 0.5 million men and women from ten diverse areas. *International Journal of COPD,* 10: 655–665.
Lakhdar R, Denden S, Mouhamed MH, Chalgoum A, Leban N, Knani J, Lefranc G, Miled, A , Chibani JB, Kheli AH 2011.Correlation of EPHX1, *GST*P1, *GSTM1,* and *GSTT1* genetic polymorphisms with antioxidative stress markers in Chronic Obstructive Pulmonary Disease. *Exp Lung Res,* 37: 195-204.
Lan Q, He X, Costa DJ, Tian L, Rothman N, Hu G, Mumford JL 2000. Indoor coal combustion emissions, *GSTM1* and *GSTT1* genotypes, and lung cancer risk: A case control study in Xuan Wei, China. *Cancer Epidemiol Biomarkers Prev,* 9: 605-608.
Lee M, Li S, Christiani DC 2010.Synergistic effects of NAT2 slow and *GSTM1* null genotypes on carcinogen DNA damage in the lung. *Cancer Epidemiol Biomarkers Prev,* 19: 1492–1497.
Lee S J, Kim S W, Kong K A, Ryu Y J, Lee J H, Chang J H 2015. Risk factors for chronic obstructive pulmonary disease among never-smokers in Korea. *Int J Chron Obstruct Pulmon Dis,* 10: 497–506.
Leonard SS, Bower JL, Shi X 2004. Metal-induced toxicity, carcinogenesis, mechanisms and cellular responses. *Mol Cell Biochem,* 255: 3-10.
Levy ML, Quanjerb PH, Bookerc R, Cooperd BG, Holmese S, Iain R 2009. Small diagnostic spirometry in primary care proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society Recommendations. *Prim Care Respir J,* 18:130-147.
Liu H, Lin M, Liu P, Chan C, Chen H 2009. Health risk assessment by measuring plasma malondialdialdehyde (MDA), urinary 8-Hydroxydeoxyguanosine (8-OH-dg) and DNA strand breakage following metal exposure in foundry workers. *J Hazard Mater,* 170: 699-704.
Liu YJ, Huang PL, Chang YF, Chen YH, Chiou YH, Xu ZL, Wong RH 2006. *GST*P1 genetic polymorphism is associated with a higher risk of DNA damage in pesticide-exposed fruit growers. *Cancer Epidemiol Biomarkers Prev,* 15: 659-666.
Lushchak VI 2012. Glutathione homeostasis and functions: Potential targets for medical interventions. *J Amino Acids,* 2012: 1- 26 .
MacNee W 2005. Pathogenesis of Chronic Obstructive Pulmonary Disease. *Proc Am Thorac Soc,* 2: 258-266.
Majo J, Ghezzo H, Cosio MG 2001. Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *Eur Respir J,* 17: 946-953.
Mak JCW, Ho SP, Yu WC, Choo KL, Chu CM, Yew WW, Lam WK, Chan-Yeung M 2007. Polymorphisms and functional activity in superoxide dismutase and catalase genes in smokers with COPD. *Eur Respir J,* 30: 684–690.
Maluf SW, Mergener M, Dalcanale L, Costa CC, Pollo T, Kayser M, da Silva LB, Pra D, Teixeira PJ 2007. DNA damage in peripheral blood of patients with Chronic Obstructive Pulmonary Disease (COPD). *Mutat Res,* 626: 180-184.

Maluf SW, Erdtmann B 2000. Evaluation of occupational risk in a Brazilian hospital. *Genet Mol Biol,* 23: 485-488.

Manar MH, Brown MR, Gauthier TW, Brown LAS 2004. Association of Glutathione-S-Transferase-P1 (*GST*-P1) polymorphisms with bronchopulmonary dyspalsia. *J Perinatol,* 24: 30-35.

Miranda-Vilela AL, Alves PCZ, Akimoto AK, Lordelo GS, Goncalves CA, Grisolia CK, Klautau-Guimaraes MN 2010. Gene polymorphism against DNA damage induced by hydrogen peroxide in leukocytes of healthy humans through comet assay: A quasi-experimental study *Environ Health*, 9: 21.

Mirzaei B, Rahmani-nia F, Salehi Z, Rahimi R, Saravani MF, Ahmadi R, Hoor ZMB 2014. Effects of GSTM1 gene polymorphism on oxidative DNA damage and lipid peroxidation following exhaustive exercise in young wrestlers. *Med Sport,* 67: 437-448.

Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, Joshi SR, Sadikot S, Gupta R, Gulati S, Munjal YP 2009.Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assoc Physicians India,* 57: 163-169.

Moretti M, Dell'Omo M, Villarini M, Pastorelli R, Muzi G, Airoldi L, Pasquini L 2007. Primary DNA damage and genetic polymorphisms for *CYP1A1, EPHX* and *GSTM1* in workers at a graphite electrode manufacturing plant. *BMC Public Health,* 7: 1-10.

Naccarati A, Soucek P, Stetina R, Haufroid V, Kumare R, Vodickova L, Trtkova K, Dusinska M, Hemminki K, Vodicka P 2006.Genetic polymorphisms and possible gene–gene interactions in metabolic and DNA repair genes: Effects on DNA damage. *Mutat Res,* 593: 22–31.

Nishigori C, Hattori Y, Toyokuni S 2004. Role of reactive oxygen species in skin carcinogenesis. *Antioxid Redox Signal,* 3: 561-570.

Park S, Lee K, Kang D, Lee K, Had E, Hong Y 2006. Effect of genetic polymorphisms of *MnSOD* and MPO on the relationship between PAH exposure and oxidative DNA damage. *Mutat Res,* 593: 108–115.

Pietras T, Szemraj J, Witusik A, Holub M, Panek M, Wujcik R, Gorski P 2010. The sequence polymorphism of *MnSOD* gene in subjects with respiratory insufficiency in COPD. *Med Sci Monit,* 16: CR427-CR432.

Rabe KF, Beghe B, Luppi F, Fabbri LM 2007. Update in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med,* 175: 1222-1232.

Rahman I, Adcock IM 2006. Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J,* 28: 219-242.

Rahman I, Kode A, Biswas SK 2007. Assay for quantitative determination of Glutathione and Glutathione Disulphide levels using enzymatic recycling. *Method Nat Protoc,* 1: 3159-3165.

Rahman K 2007. Studies on free radicals, antioxidants, and co-Factors. *Clin Interv Aging,* 2: 219-236.

Sambrook J, Russell DW 2001. *Molecular Cloning: A Laboratory Manual.* 3rd Edition. New York: Cold Spring Harbor Laboratory Press. ISBN 978-0-87969-577-4.

Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M 2000. Upregulation of Gelatinases A and B, Collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest,* 117: 684-694.

Sellappa S, Prathyumnan S, Balachandar V 2010. DNA Damage induction and repair inhibition among building construction workers in South India. *Asian Pac J Cancer Prev,* 11: 875-880.

Siedlinski M, Diemen CCV, Postma DS, Vonk JM, Boezen HM 2009.Superoxide Dismutase, lung function and bronchial responsiveness in a general population. *Eur Respir J,* 33: 986-992.

Sin DD, Anthonisen NR, Soriano JB, Agustí AG 2006. Mortality in COPD: Role of comorbidities. *Eur Respir J,* 28: 1245-1257.

Singh S, Kumar V, Singh P, Banerjee BD, Rautela RS, Grover SS, Rawat DS, Pasha ST, Jain SK, Rai A 2012. Influence of CYP2C9, *GSTM1, GSTT1* and NAT2 genetic polymorphisms on DNA damage in workers occupationally exposed to organophosphate pesticides. *Mutat Res,* 741: 101-108.

Siu ST, Udaltsova N, Iribarren C, Klatsky A 2010. Alcohol and lung airways function. *Perm J,* 14: 11-17.

Sivacoumar R, Jayabalou R, Swarnalatha S, Balakrishnan K 2006. Particulate matter from stone crushing industry: Size distribution and health effects. *J Environ Eng,* 132: 405-414.

Sobti RC, Bhardwaj DK 1991. Cytogenetic damage and occupational exposure. I. Exposure to stone dust. *Environ Res,* 56: 25–30.

Shiota S, Ichikawa M, Suzuki K, Fukuchi Y, Takahashi K 2015. Practical surrogate marker of pulmonary dysanapsis by simple spirometry: An observational case–control study in primary care. *BMC Family Practice,* 16:1-8.

Their R, Brüning T, Roos PH, Rihs HP, Golka K, Ko Y, Bolt HM 2003. Markers of genetic susceptibility in human environmental hygiene and toxicology: The role of selected CYP, NAT and *GST. Int J Hyg Environ Health,* 206: 149-171.

Tiwari R, Sathwara NG, Habibullah S 2004. Serum copper levels among quartz stone –crushing workers: a cross sectional study. *Indian J Physiol Pharmacol,* 48: 337–342.

Tiwari RR, Sharma YK, Saiyed HN 2011. Tuberculosis among workers exposed to free silica dust. *Indian J Occup Environ Med,* 11: 61-64.

Tug T, Karats F, Terzi SM, Ozdemir N 2005. Comparison of serum malondialdehyde levels determined by two different methods in patients with COPD: HPLC or TBRAS methods. *Lab Med,* 36: 41-44.

Turner MC, Chen Y, Krewski D, Calle EE, Thun MG 2007. Chronic Obstructive Pulmonary Disease is associated with lung cancer mortality in a prospective study of never smokers. *Am J Respir Crit Care Med,* 176: 285-290.

Tzortzaki EG, Dimakou K, Neofytou E, Tsikritsaki K, Samara K, Avgousti M, Amargianitakis V, Gousiou A, Menikou S, Siafakas NM 2012. Oxidative DNA damage and somatic mutations: A link to the molecular pathogenesis of chronic inflammatory airway diseases. *Chest,* 141: 1243-1250.

Ugbogu OC, Ohakwe J, Foltescu V 2009. Occurrence of respiratory and skin problems among manual stone-quarrying workers. *Mera: Afr J Respir Med*, 23-26.

Valavanidis A, Vlachogianni T, Fiotakis C 2009. 8-Hydroxy-2' -Deoxyguanosine (8-Ohdg): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health Part C*, 27: 120-139.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 39: 44-84.

Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR, McLaurin JL, Khan A, Teass A 1995. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: Potential role of free radicals. *Am J Respir Crit Care Med*, 152: 1003-1009.

Valverda M, Trijo C, Rajoo E 2001. Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA metal interaction? *Mutagenesis*, 16: 265–270.

Vedyakov AM, Tonevitskii AG 2006. Analysis of a series of significant genetic polymorphisms in athletes. *Hum Physiol*, 32: 204–208.

Vettriselvi V, Vijayalakshmai K, Paul SFD, Venkatachalam P 2006. Genetic variation of *GSTM1*, *GSTT1* and *GST*P1 genes in South Indian population. *Asian Pacific J Cancer Prev*, 7: 325-328.

Villarini M, Moretti M, Fatigoni C, Agea E, Dominici L, Mattioli A, Volpi R, Pasquini R 2008. Evaluation of primary DNA damage, cytogenetic biomarkers and genetic polymorphisms for CYP1A1 and *GSTM1* in road tunnel construction workers. *J Toxicol Environ Health*, 71: 430-439.

Wang LI, Miller DP, Sai Y, Liu G, Su L, Wain JC, Lynch TJ, Christiani DC 2001.Manganese Superoxide Dismutase Alanine-to-Valine polymorphism at codon 16 and lung cancer risk. *J Natl Cancer Inst*, 93: 1818–1821.

Weiner JS, Lourie JA 1981. *Human Biology: A Guide to Field Methods. Practical Human Biology.* New York: Academic Press, Inc.

WHO expert consultation 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *The Lancet*, 363: 157-63.

Yang IA, Relan V, Wright CM, Davidson MR, Sriram KB, SMS Francis, Clarke BE, Duhig EE, Bowman RV, Fong KM 2011. Common pathogenic mechanisms and pathways in the development of COPD and lung cancer. *Expert Opin Ther Targets*, 15: 439-456.

Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG 2000.Genetic susceptibility to Chronic Obstructive Pulmonary Disease in Koreans: Combined analysis of polymorphic genotypes for Microsomal Epoxide Hydrolase and Glutathione S-Transferase M1 and T1. Thorax, 55: 121-125.

Yoshida T, Tuder RM 2007. Pathobiology of cigarette smoke-induced Chronic Obstructive Pulmonary disease. *Physiol Rev*, 87: 1047-1082.

Young RP, Hopkins RJ, Christmas T, Black PN, Metcalf P, Gamble GD 2009. COPD prevalence is increased in lung cancer, independent of age, sex and smoking history. *Eur Respir, J*, 34: 380-86.

Young RP, Hopkins RI, Gamble GD, Etzel C, El-Zein R, Crapo JD 2011a. Genetic evidence linking lung cancer and COPD: A new perspectvive. *The Application of Clinical Genetics*, 4: 99-111.

Young RP, Hopkins RJ, Hay BA, Gamble GD 2011b. *GSTMI* null genotype in COPD and lung cancer: Evidence of a modifier or confounding effect? *The Application of Clinical Genetics*, 4: 137-144.

• idzik J, Slabá E, Joppa P, Kluchová Z, Dorková Z, Skyba P, Habalová V, Šalagoviè J, Tkáèová R 2008. Glutathione S-Transferase and Microsomal Epoxide Hydrolase gene polymorphisms and risk of Chronic Obstructive Pulmonary Disease in Slovak population. *Croat Med J*, 49: 182-191.