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RESEARCH ARTICLE

Assessment of 8-oxo-7, 8-dihydro-2′-deoxyguanosine and malondialdehyde levels as oxidative stress markers and antioxidant status in non-small cell lung cancer

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Abstract

Objective: The present investigation was taken up to evaluate the 8-oxo-7,8-dihydro-2′-deoxyguanosine and malondialdehyde as markers of oxidative stress, the levels of antioxidants and the correlations between these oxidative stress markers and antioxidants in lung cancer patients.

Methods: The study included 222 patients (158 men and 64 women, age ranging from 32 to 85 years) and 207 control subjects (153 men and 54 women, aged 30–80 years) for the analysis of urinary excretion of 8-oxodG using an ELISA assay, plasma malondialdehyde using spectrophotometer and red cell Cu-Zn SOD and GPx activities by kit methods.

Results: The levels of 8-oxodG and malondialdehyde were significantly higher ($p < 0.001$) and red cell superoxide dismutase and glutathione peroxidase activities ($p < 0.001$) were significantly lower in lung cancer patients than in controls. There was a significantly positive correlation between 8-oxodG and malondialdehyde ($r = 0.912$, $p < 0.001$) and a negative correlation between 8-oxodG and antioxidants.

Conclusions: Our results demonstrate that an increased rate of oxidative stress might play a role in the pathogenesis of lung cancer as evidenced by a failure in the oxidant/antioxidant balance in favour of lipid peroxidation and DNA damage.

Keywords: Oxidative stress, non-small cell lung cancer, oxidant/antioxidant balance, malondialdehyde (MDA), 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)

Introduction

Lung cancer is a common cause of death in India and other countries across the globe (1.38 million deaths annually) mainly due to late presentation. Lung cancer accounts for 9.4% of all male cancers and 2.7% of all female cancers in India. Incidence of lung cancer has increased in India during the last few years (Namrata et al. 2009). Smoking, passive smoking and exposure to environmental chemical pollutants have been attributed to the increasing incidence of lung cancer in India (Behera & Balamugesh 2004). In Andhra Pradesh the increased incidence of lung cancer is attributed to increased air pollution due to automobile exhaust (Sreedevi et al. 2009) and industries (in urban areas) and excessive pesticide usage (in rural areas) (Kiranmai et al. 2010).

Lung is a primary organ with large surface area directly exposed to ambient air and therefore higher oxygen tensions and thus a target for oxidative stress. The local oxygen partial pressure at the alveolar level is much higher when compared to other vital organs. Oxygen pressure in the inhaled air is 20 kPa (150 mm Hg) and is ~13.3 kPa (100 mm Hg) at the alveolus, but it is ~6 kPa (45 mm Hg) in the blood and may be as low as 1 mm Hg in some sites within other organs (Vuokko et al. 2003). Oxygen tension

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is known to regulate Reactive oxygen species (ROS) production (Wu et al. 2007) and increases lung microvascular permeability. Hyperoxia increases both mitochondrial and microsomal oxidant production in lung cells, and physiologic studies demonstrate that increased pulmonary microvascular permeability is an early indicator of oxygen injury (Hazinski et al. 1998).

Exogenous free radical–generating environmental irritants and pollutants including oxidants such as cigarette smoke, ozone, and endogenous factors like inflammation and activation of inflammatory cells contribute to free radical generation (Vuokko et al. 2003). ROS (singlet oxygen molecule ($^1O_2$), superoxide radical (O_2^-), hydroxyl radical (OH) and peroxynitrite anion ($ONOO^-$)) can initiate lipid peroxidation or can attack all nucleobases of DNA and also the dNTP pool (and RNA) (Dizdaroglu et al. 2002) resulting in oxidatively generated DNA damage leading to mutagenesis, carcinogenesis and cell death if antioxidant system or the DNA repair mechanisms are impaired or inadequate. Lipid peroxidation products include conjugated dienes and malondialdehyde (MDA) which may also react with DNA and cause oxidative modifications. One of the oxidation products of DNA, 8-oxodG, is produced by oxidation of 2′-deoxyguanosine in DNA.

Earlier reports showed increased urinary excretion of 8-oxodG in patients with prostate, bladder, breast and lung cancers (Miyake et al. 2004; Wu et al. 2004; Kuo et al. 2007; Caliskan et al. 2008). Reports on the effect of stage of cancer and smoking on excretion of urinary 8-oxodG are conflicting. While some studies have shown that increased levels of 8-oxodG in urine was associated with advanced stage of cancer (Honda et al. 2000; Caliskan et al. 2008), Yano et al. (2009) and Kuo et al. (2007) did not observe any such association. Some researchers showed that smokers excrete more 8-oxodG in urine than non-smokers indicating that cigarette smoking is a major cause of oxidatively generated DNA damage (Loft et al. 1992; Yun-Chul et al. 2000; Yano et al. 2009). Some others did not observe any difference in 8-oxodG levels between smokers and non-smokers (Gackowski et al. 2005; Caliskan et al. 2008). Changes in enzymatic and non-enzymatic antioxidant systems and thiobarbituric acid (TBA) reactive products have been described in various cancers including lung cancer (Uzun et al. 2000; Aymelek et al. 2006). Caliskan et al. (2008) and Esme et al. (2008) reported elevated MDA levels in lung cancer patients. Chung-man et al. (2001) evaluated antioxidant expression in non-small cell lung cancer (NSCLC) and showed significantly increased levels of superoxide dismutase (SOD) and similar glutathione peroxidase (GPx) activities in cancerous tissue when compared with tumor free lung tissues. Kaynar et al. (2005) reported increased MDA levels, red cell Cu-Zn SOD activities and apparently increased GPx activities in NSCLC. However some others showed decreased levels of red cell SOD and GPx activities in lung cancer patients (Gromadzinska et al. 2003).

In the view of the above, and limited reports on lung cancer patients in the present day, especially from India, in the context of increased pollution in urban and rural areas of AP, the present study was under taken to evaluate urinary excretion of 8-oxodG, levels of MDA in plasma and red cell Cu-Zn SOD and GPx activities in lung cancer and the correlations between these oxidative stress markers and antioxidants in lung cancer patients.

Material and methods

Patient selection and study design

The present study included 222 patients with histologically or cytologically confirmed diagnosis of primary Non-small cell lung cancer (newly diagnosed and previously untreated) recruited from the Indo-American cancer Hospital, Hyderabad, India during the period, June 2006–January 2008. The revised lung cancer staging system for the classification of cases was used (Greene et al, 2002). A total of 207 age and sex-matched healthy controls (apparently healthy and without a history of cancer and any other chronic diseases) from the general population of same geographic region were selected for comparison. The study was carried out with the approval of ethics committee, Indo-American cancer hospital, Banjara Hills, Hyderabad. Informed and educated consent was taken from all Lung cancer patients and healthy controls. Data on clinical findings, stage of the disease etc of lung cancer patients, socio-demographic characteristics such as age, sex, lifetime occupational history, family history of cancer, smoking status (Non-smokers, ex-smokers and current smokers), number of cigarettes per day and duration of smoking, alcohol consumption etc were collected using a standard questionnaire. Smokers were considered as current smokers at the time of diagnosis. Ex-smokers were those people who had smoked at least 100 cigarettes in their life time. Smoking status of the subjects was calculated as the average tobacco consumption expressed in pack years. Pack years were computed as the number of cigarettes smoked per day multiplied by the duration of smoking in years.

Blood and urine collection

10 mL peripheral blood in heparin and 24 h urine samples were collected from patients and control subjects. Urine samples were centrifugation at 3000×g for 10 min to remove particulate matters and stored at –70°C for further analysis. The blood samples were centrifuged at 1600x g for 10 min to separate plasma and Red blood cells and stored at –70°C for subsequent assays of MDA and antioxidant activities. Red blood cell lysate was prepared by washing packed red blood cells three times with cold normal saline under centrifugation at 3000×g, cells followed by lysis with four volumes of cold deionized water.

Analysis of 8-oxodG

Urinary excretion of 8-oxodG was evaluated in 125 patients and 100 controls. The concentration of 8-oxodG in urine samples was measured using a competitive

in-vitro enzyme-linked immunosorbent assay (ELISA) kit method (Japan Institute for the Control of Aging, Shizuoka, Japan) described previously (Yano et al. 2009). Briefly the protocol involves thawing of urine samples and addition of fifty microlitres of primary monoclonal antibody and 50 μL of sample or standard to microtitre plates, precoated with 8-oxodG. The plates were sealed tightly, incubated at 37°C for 1 h, followed by a wash with 250 μL phosphate buffered saline (PBS). 100 μL of secondary antibody conjugated to horse radish peroxidase was then added to each well, incubated and washed. 100 μL of enzyme substrate was then added to each well, and the reaction was stopped by addition of 100 μL 1 N phosphoric acid. Absorbance readings were taken with a spectrophotometer at 450 nm. The amount of 8-oxodG in each subject was calculated by comparison with a standard curve. Urinary creatinine was measured according to the method of Taussky (1954). The level of 8-oxodG in urine was expressed as nanograms per milligram creatinine.

Lipid peroxidation

Lipid peroxidation products were quantified in plasma of 222 lung cancer patients and 204 controls using TBA method (Gavino et al. 1981) which is a simple and rapid technique. MDA is formed as an end product of lipid peroxidation which reacts with TBA reagent under acidic conditions to generate a pink colored product. 0.5 mL of plasma was made up to 1 mL with saline. An equal volume of trichloroacetic acid (TCA) was added and incubated at 37°C for 20 min, and centrifuged at 500g. To 1 mL of TCA extract (the supernatant) 0.25 mL of TBA was added and heated in a water bath at 95°C for 1 h till a faint pink color appeared. After cooling the color was extracted in 1 mL butanol and the intensity was read at 532 nm. 1,1,3,3 tetra ethoxypropane (1–100 nmol/mL) was used as the standard.

Estimation of antioxidant enzymes

Red cell Cu – ZnSOD and GPx activities were estimated in 207 patients and 207 control samples using SOD-525 and GPx-340 spectrophotometric assay kits (Bioxytech; OXIS International, Portland, U.S.A) respectively. Haemoglobin (Hb) concentrations were assayed by a commercially available kit (Sigma, St. Louis, MO, USA).

Estimation of Cu–Zn SOD

The assay is based on the SOD-mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm (Lisa et al. 2001).The upper phase of erythrocyte suspension was collected after addition of ice-cold extraction reagent with ethanol/chloroform (62.5/37.5 v/v). The assay sample consisted of 900 μL of buffer, 40 μL sample and 30 μL of 1-methyl-2-vinylpyridinium followed by incubation at 37°C for 1 min. Finally 30 μL of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c] fluorene was added, vortexed briefly and spectrophotometrically measured at 525 nm. Erythrocyte Zn/Cu-SOD activity was expressed as units per gram of Hb.

Estimation of GPx

GPX activity was measured in 207 patients and 207 controls. Total GPx activity was determined spectrophotometrically in the red cell lysate via an indirect coupled assay (Paglia & Valentine, 1967). Prior to assay the sample was diluted into Assay Buffer. 350 μL Assay buffer, 350 μL NADPH Reagent and 70 μL samples were pipetted into cuvette in the spectrophotometer. Then 350 μL of dilute tert-Butyl Hydroperoxide was added to cuvette and mixed thoroughly. Readings were recorded and the change in A340 for three minutes was observed. The first 15 sec of the reaction was excluded from data analysis as the rates may not be representative of the enzyme activity due to sample mixing. The delta A340/min for the sample is converted to NADPH consumed and expressed as units per gram of Hb.

Statistical analysis

Mean and standard deviation was calculated for each biomarker. The significance of the differences between controls and patients end point means were analyzed using Student's t test. ANOVA (analysis of variance) was used for comparisons among the three or more groups. Multiple regression analysis was used to investigate the relations between continuous variables and the log transformed concentrations of urinary 8-oxodG. Multiple linear regression analysis was performed to assess the association between 8-oxodG and the independent variables. Pearson correlation analysis was used for testing relationships between 8-oxodG, MDA and antioxidant enzyme activities. The results were considered to be significant at p values of less than 0.05. All calculations were performed using SPSS program (Version 15.0 for Windows 98).

Results

The study subjects comprised of 222 lung cancer patients (aged 30– 85 years) and 207 healthy controls (aged 30– 80 years). Smoking is known to be an important risk factor in the etiology of lung cancer. Passive smokers are also known to be at risk for development of lung cancer. In the view of this, patients and controls were categorized into smokers, ex-smokers and non-smokers based on their smoking habit. Lung cancer was predominantly seen among males affecting older men and predominantly included current smokers (45.95%) who have less than 40 pack-years (31.08%). Surprisingly apparently equal number of non-smokers (40.54%) were affected with lung cancer. 48.65% of lung cancer had adenocarcinoma while 43.24% had squamous cell carcinoma. Most of the patients were in advanced stages of cancer - stage IV (58.11%) followed by stage III (34.23%), stage II (7.66%)

and none were found in stage I of lung cancer at the time of diagnosis (Table 1).

Evaluation of urinary excretion of 8-oxodG (ng/mg creatinine) in lung cancer patients indicated that the excretion rate was significantly higher in patients (6.06 ± 0.81) when compared with the controls values (4.36 ± 0.69) and increased with progression of stage of lung cancer especially across stages III and IV ($p < 0.001$). Although there was an apparent increase in 8-oxodG excretion in stage II, it did not show significance (Table 2). There was no significant difference in the creatinine levels between the patients and controls (0.92 vs 1.01 mg/dL, $p = 0.1$) The values of lipid peroxidation products (n.mol MDA eq) were significantly increased in patients when compared to the control values and increased progressively with the advancement of stage of disease. Red cell SOD and GPx activities (U/g Hb) significantly decreased in the lung cancer patients when compared with the controls. Further with increase in stage of the disease (stage II, III, IV) the reduction in SOD and GPx activities was more pronounced. However SOD did not achieve the statistical significance in stage II (Table 2).

Evaluation of 8-oxodG in patients and controls based on the habit of smoking indicated an increase in 8-oxodG levels from non-smokers to smokers. With increase in the use of cigarettes and thereby exposure to tobacco smoke (both environmental or passive and mainstream) significant increase in levels of lipid peroxidation products was seen in both patient as well as control group. Red cell SOD and GPx activities decreased in the order of non-smokers followed by ex-smokers and smokers and was found to be significant only in the smoker category of patients when compared with the controls (Table 3).

In the multiple regression analysis of 8-oxodG with other measured parameters, age and sex were found to be non-significant predicting factors for urinary 8-oxodG excretion. Smoking and stages (III and IV) of lung cancer were the strongest predictors of 8-oxodG excretion ($p < 0.001$, Table 4).

The Pearson correlation showed a positive correlation between 8-oxodG and MDA ($r = 0.912$, $p < 0.001$). A negative correlation was observed in 8-oxodG in relation to

Table 1. General characteristics of the study group.

Variables	Patients $n = 222$ (%)	Controls $n = 207$ (%)
Gender		
Male	158 (71.17)	153 (73.91)
Female	64 (28.82)	54 (26.09)
Age		
Mean ± (SD)	57.64 ± 9.95	57.26 ± 9.40
Range	32–85	30–80
Smoking Status		
Never-Smokers	90 (40.54)*	143 (69.08)
Ex-smokers	30 (13.51)*	9 (4.35)
Current Smokers	102 (45.95)*	55 (26.57)
<40 Pack Years	69 (31.08)	39 (18.85)
>40 Pack Years	33 (14.86)	16 (7.72)
Histology		
Squamous-cell carcinoma	96 (43.24)	
Adenocarcinoma	108 (48.65)	
Large cell and others	18 (8.11)	
Stages		
II	17 (7.66)	
III	76 (34.23)	
IV	129 (58.11)	

*$p < 0.001$ compared to controls.

Table 2. Mean concentration of urinary 8-oxodG, MDA and antioxidants in controls and patients based on stage of the disease (TNM system).

Parameters	Controls	Lung cancer	Stage II	Stage III	Stage IV
8-oxodG (ng/mg creatinine)	4.36 ± 0.69 (100)	6.06 ± 0.81[a] (125)	4.93 ± 0.6 (10)	5.50 ± 0.51[a] (43)	6.54 ± 0.55[a] (72)
MDA (n.mol MDA eq)	1.80 ± 0.47 (204)	4.45 ± 1.16[a] (222)	2.68 ± 0.81[a] (17)	3.51 ± 0.71[a] (76)	5.23 ± 0.63[a] (129)
GPx (U/g Hb)	54.68 ± 7.18 (207)	31.59 ± 11.18[a] (207)	51.31 ± 5.57[a] (16)	39.43 ± 7.64[a] (72)	24.2 ± 5.83[a] (119)
SOD (U/g Hb)	1152.56 ± 146.08 (207)	900.38 ± 155.20[a] (207)	1125 ± 62.60 (16)	1006.38 ± 87.45[a] (72)	806.05 ± 119.50[a] (119)

The values in the parenthesis indicates the number of subjects.
[a]Compared with controls, $p < 0.001$.

Table 3. Mean concentration of urinary 8-oxodG, MDA and antioxidants in controls and patients according to smoking status.

Parameter	Group	Smoking Status			Total
		Non-smokers	Ex-smokers	Smokers	
8-oxodG (ng/mg creatinine)	Lung Cancer	5.74 ± 0.72 (49)	5.89 ± 0.76 (20)	6.39 ± 0.78[a] (56)	6.06 ± 0.81[a] (125)
	Control	4.15 ± 0.63 (67)	4.2 ± 0.67 (7)	4.95 ± 0.49 (26)	4.36 ± 0.69 (100)
MDA (n.mol MDA eq)	Lung Cancer	4.09 ± 1.11[a] (90)	4.18 ± 1.18[a] (30)	4.84 ± 1.09[a] (102)	4.45 ± 1.16[a] (222)
	Control	1.57 ± 0.28 (40)	1.83 ± 0.31 (9)	2.42 ± 0.32 (55)	1.81 ± 0.47 (204)
GPx (U/g Hb)	Lung Cancer	35.33 ± 11.19 (83)	34.32 ± 11.95 (28)	27.56 ± 9.57[a] (96)	31.59 ± 11.18[a] (207)
	Control	57.06 ± 6.62 (143)	53.44 ± 7.19 (9)	48.69 ± 4.70 (55)	54.68 ± 7.18 (207)
SOD (U/g Hb)	Lung Cancer	967.71 ± 114.45 (83)	948.57 ± 177.34 (28)	828.12 ± 148.55[a] (96)	900.38 ± 155.20[a] (207)
	Control	1198.88 ± 135.14 (143)	1135.55 ± 134.08 (9)	1034.90 ± 119.45 (55)	1152.56 ± 149.08 (207)

The values in the parentheses indicates the number of subjects
[a]Compared with controls, $p < 0.001$.

Table 4. Multiple linear regression analysis of the urinary 8-oxodG and other covariates.

Covariates	*B*	S.E	*t*	95% CI Lower	95% CI Upper	*P* Value
Age	0.004	0.004	1.068	−0.003	0.011	0.28
Sex	0.192	0.09	2.118	0.013	0.371	0.075
Smoking	0.428	0.045	9.508	0.339	0.517	<0.001
Stages						
Stage II	0.061	0.128	0.475	−0.19	0.31	0.63
Stage III	0.33	0.05	6.821	0.24	0.43	<0.001
Stage IV	0.631	0.027	23.27	0.57	0.68	<0.001

Table 5. Pearson correlation coefficients between oxidative stress markers (8-oxodG and MDA) and antioxidant enzyme activities in the patients.

Parameter	8-oxodG *r*	8-oxodG *P* value	MDA *r*	MDA *P* value
MDA	0.912**	<0.001		
SOD	−0.799**	<0.001	−0.851**	<0.001
GPx	−0.875**	<0.001	−0.929**	<0.001

**Correlation is significant at 0.01 level (two-tailed).

SOD ($r = -0.799$, $p < 0.001$) and GPx ($r = -0.875$, $p < 0.001$). A significantly negative correlation of MDA with antioxidant enzymes was also noted (SOD $r = -0.851$, $p < 0.001$; GPx $r = -0.929$, $p < 0.001$ respectively, Table 5).

Discussion

Cigarette smoking is known risk factor for lung cancer (Vineis et al. 2004). Cigarette smoke is a complex mixture containing different pyridine alkaloids such as nicotine, ammonia, acrolein, phenols, acetaldehyde, N-nitrosamine; polycyclic aromatic hydrocarbons (PAH) such as benzopyrine; combustion gases such as carbon monoxide, nitrogen oxides, hydrogen cyanide, trace metals, α-emitter radioactive elements like polonium, radium and thorium (Koul et al. 2001). Among the multiple components of tobacco smoke, 20 carcinogens convincingly cause lung tumors in laboratory animals or humans and are, therefore, likely to be involved in lung cancer induction. Of these, PAH and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone are likely to play major roles (Hecht 1999). PAH entail metabolic activation to exert their carcinogenic effects, and one vital pathway proceeds through a three-step sequence resulting in the formation of diol epoxides, which react with DNA to produce adducts that can cause mutations and initiate the carcinogenic process. The formation of a PAH diol epoxide occurs rapidly in smokers (Zhong et al. 2011). It is estimated that a single cigarette puff may contain approximately, 10^{14} free radicals in tar phase and 10^{15} radicals in the gas phase causing increased generation of ROS (Dilyara et al. 2007).

Free radical induced lipid peroxidative tissue damage and oxidatively generated DNA damage have been implicated in the pathogenesis of various diseases including cancers. Free radical measurement directly *in-vivo* is not possible and it is imperative to rely on the quantification of their reaction products such as proteins, carbonyls, modified DNA, and lipid peroxidation products (Karatas et al. 2002. Evidences indicate that urinary 8-oxodG is produced by the activity of selected members of the Nudix hydrolase family of enzymes, clearing the deoxyribonucleotide pool via the degradation of 8-oxo-7,8-dihydro-2′-deoxyguanosine-5′-triphosphate and 8-oxo-7,8-dihydro-2′-deoxyguanosine-5′-diphosphate, yielding mononucleotide products that can then be de-phosphorylated to 8-oxodG and excreted. However, nucleotide excision repair (NER), transcription-coupled repair, nucleotide incision repair (NIR), mismatch repair and various exonuclease activities, such as proofreading function associated with DNA polymerases, can also possibly generate initial products that could yield 8-oxodG after further metabolism (Evans et al. 2010).

The present study was initiated in the year 2006. Evaluation of 8-oxodG was adopted after reviewing the literature (Toyokuni et al. 1997; Yun-Chul et al. 2000; Shimoi et al. 2002). Both HPLC and ELISA techniques have been recommended for the assessment of the levels of 8-oxodG. ELISA method is a simple, rapid technique with high throughput, more economical and acceptable method. Early studies have assumed the measurement of urinary 8-oxodG by ELISA to be an appropriate method as its results would be in concordance with results of chromatographic methods, reported associations and group differences. Pathological diseases that involve oxidative stress and therefore a larger response in urinary 8-oxodG should exhibit differences more readily detected by ELISA. Subsequently the latest reports have endorsed the reasons for popularity of ELISA to be its cost effectiveness and its applicability in large scale samples. However the authors have suggested that HPLC is the gold standard because of its sensitivity, reproducibility and accuracy. HPLC technique is highly specialized and needs special equipment, reagents, expensive columns, trained labor and also requires some pre treatment of the urine specimens (Garratt et al. 2010).

Although several studies described that commercial 8-oxodG ELISA kits correlate sufficiently with HPLC techniques to be an easier alternative for laboratories without access to gold-standard techniques, the assumption that significant correlation translates into a similar ability to differentiate disease categories or treatment groups was considered to be not convincing. Garret et al. (2010) measured urinary 8-oxodG and creatinine concentrations in cystic fibrosis patient using LC–MS/MS and two variants of a commercial ELISA, compared the results and showed that, despite significant correlation, both ELISAs overestimated the levels of 8-oxodG, and neither ELISA accurately depicted the difference in group means that was observed by gold-standard LC–MS/MS. Further the authors showed that N45.1 antibody used in ELISA also binds to urea found in vivo, instead of 8-oxodG on initial incubation

temperatures 37°C/4°C and suggested that pretreatment of samples with urease and incubation at 4°C may bring the ELISA results comparable to chromatographic techniques. They endorsed that chromatographic techniques, despite their cost and complexity, remained the gold standard in urinary 8-oxodG assessment. Song et al. (2009) have demonstrated cross reactivity of N45.1 antibody (used in ELISA) with urea, instead of 8-oxodG, contributing to false positive results by ELISA and suggested that pretreatment of samples with urease and incubation at 4°C may bring the ELISA results comparable to chromatographic techniques.

In the current study, 8-oxodG levels were measured using commercial ELISA kit using the antibody N45.1 with incubation at 37°C. The observed increased levels of 8-oxodG seen in lung cancer patients when compared to controls and with the progression of the disease especially with stage III and IV was consistent with the previous reports (Yano et al. 2009). Further the present study also corroborated the previous studies (Yano et al. 2009; Yun-Chul et al. 2000) which demonstrated a significant increase in urinary 8-oxodG in smokers when compared to non-smokers. Using HPLC also increased levels have been described in lung cancer with progression of the disease (Loft et al. 2006; Caliskan et al. 2008).

ROS can react with polyunsaturated lipids initiating a self-perpetuating chain reaction of lipid membranes and can induce formation of lipid peroxidation products, such as peroxyl radical, conjugated diens, 4-hydroxynonenal (HNE) and also MDA (Marnett 2000). MDA, one of the well-known secondary products of lipid peroxidation may be used as an indicator of cell membrane injury. Early reports have measured MDA by a simple rapid and inexpensive 2-thiobarbituric acid (TBA) method which can be applied when studying large number of samples. This method involves the reaction of TBA with MDA in biomaterials, which can then be detected spectrophotometrically at 532–535 nm. However TBA method lacks sensitivity as well as specificity. The reaction of TBA with products of lipid peroxidation such as hydroperoxides and conjugated aldehydes interferes with TBA, which results in a lack of sensitivity of the method, Further these TBA reactive compounds formed due to side reactions absorb near 535 nm similar to that of MDA-$(TBA)_2$ making the assay less specific. The gold standard for measurement of MDA is HPLC method because of its high analytical sensitivity and specificity. In HPLC diaminonapthalene (DAN) is used instead of TBA to form a DAN–MDA complex and there are no condensing TBA compounds to interfere with other substances (Karatas 2002).

The lung contains intracellular antioxidant enzymes such as superoxide dismutase (SOD), catalase and the glutathione system (reduced glutathione (GSH) + glutathione peroxidase (GPx) to maintain a normal redox state. Large amounts of GSH, GPx, SOD and catalase are also found in epithelial lining fluid (ELF) along with ceruloplasmin, transferrin, vitamins C and E, ferritin, other serum proteins and small molecules, like bilirubin (Comhair & Erzurum 2002). SOD is the first enzyme in the antioxidant defense that catalyses the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) that can be transformed into H_2O and O_2 by catalase. The quinone-semiquinone radicals from the tar phase of cigarette smoke are capable of reducing molecular oxygen to superoxide radicals and ultimately can inactivate SOD activity (Anbarasi et al. 2006). Cigarette smoke has also been shown to be associated with depletion of some antioxidants including vit C, α-tocopherol, carotenoids, glutathione-S-transferase and GPx (Liu et al. 2002; Wei et al. 2001). Currently commercial kits are being used for the estimation of SOD and GPx activities in human subjects, in large sample size cost effectively, mainly for its precision and reproducibility.

In the present study the observed increased MDA levels and reduced SOD and GPx activities in lung cancer patients when compared to the controls and with the progression of the disease as well as in smokers when compared to non-smokers endorse the fact that oxidative stress induces lung cancer. The results were parallel to the previous findings of Esme et al. (2008); Tsao et al. (2007); Gramadzinska et al. (2003); Ho et al. (2007); Gupta et al. (2010).

Intracellular aldehydes, such as HNE or MDA, can directly react with DNA (Marnett 2000) reducing DNA repairing capacity and leading to mutagenesis and carcinogenesis (Feng et al. 2006). Studies have demonstrated that 8-oxodG formation may be related to intracellular lipid peroxidation (Wong et al. 2006) where as Bergman et al. (2004) demonstrated that 8-oxodG and MDA may behave quite differently during oxidative stress. In the current study correlation between 8-oxodG and MDA levels in lung cancer patients showed a positive association reflecting the effect of lipid peroxidation on DNA damage. Similar findings have been described previously (Caliskan et al. 2008; Perez et al. 2002). Since some reports correlated 8-oxodG levels with antioxidants (vitamins) an attempt was made to correlate antioxidant enzyme activities (SOD & GPx) with oxidatively generated DNA damage and MDA levels. The observed negative correlation between urinary 8-oxodG levels and SOD and GPx activities can be attributed to lowered O_2^- scavenging activity of SOD. Though several ROS induce DNA damage, O_2^- is the major reactive species, which can selectively damage guanine base of DNA and responsible for the formation of 8-oxodG (Cadet et al. 2003). Similarly MDA levels negatively correlated with SOD and GPx in the current study. Malondialdehyde cross-linking and lipid peroxidation have been suggested to play a role in immunological destruction of plasma and erythrocyte antioxidants (Nishino et al. 2006).

Studies on oxidative stress biomarkers in lung cancer patients are limited. To the best of our knowledge this is the first study which investigated this aspect and also

presented correlation of MDA and antioxidant enzyme activities (SOD & GPx) with oxidatively generated DNA damage.

In conclusion, our results demonstrate that an increased rate of oxidative stress might play a role in the pathogenesis of lung cancer and its progression as evidenced by a failure in the oxidant/antioxidant balance in favour of lipid peroxidation and DNA damage. The positive correlation between 8-oxodG and MDA and a negative correlation of MDA with SOD and GPx may reveal an effect of lipid peroxidation on DNA damage and antioxidant defense. Further studies are warranted considering the possible binding of N45.1 antibody (used in ELISA) with urea instead of 8-oxodG in vivo, pretreatment of samples with urease, and comparative studies of ELISA by incubation at 37°C/4°C and HPLC results to define the status of 8-oxodG.

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Declaration of interest

The authors report no declarations of interest.

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