Original Article

Smoking intensity, oxidative stress and chemotherapy in non-small cell lung cancer: A correlated prognostic study

Abhilasha Gupta[1], Shruti Srivastava[1], Rajendra Prasad[2], Shanker M. Natu[1], Balraj Mittal[3], Mahendra P. S. Negi[4], Anand N. Srivastava[1,*]

[1] *Department of Pathology, Chhatrapati Shahuji Maharaj Medical University, (C.S.M.M.U) Lucknow, India;*
[2] *Department of Pulmonary Medicine, Chhatrapati Shahuji Maharaj Medical University, (C.S.M.M.U) Lucknow, India;*
[3] *Department of Genetics , Sanjay Gandhi Post Graduate Institute of Medical Sciences, (S.G.P.G.I.M.S) Lucknow, India;*
[4] *Division of Biometry and Statistics, Central Drug Research Institute (C.D.R.I), Lucknow, India.*

Summary **Cigarette smoking is a well known environmental risk factor for lung cancer; furthermore it can also enhance lung carcinogenesis by free radical mediated reactions. In addition smoking affects the rates of metabolism of several drugs and may contribute to poor cancer survival. The purpose of the present work, therefore, was to see the relationship of different smoking intensities with oxidative stress and survival after platinum based chemotherapy in non-small cell lung cancer (NSCLC). The oxidative stress levels (LPO, NO, SOD, and GSH) of 144 control subjects and 203 advanced stage NSCLC patients were assessed at day '0', after the 3rd and 6th cycle of chemotherapy. Pack year (PY) was stratified in groups (1-20, 21-50, > 50) for further analysis. Groups were compared using repeated measured ANOVA, while survival curves were compared by Kaplan-Meier methods. Oxidative stress levels of smokers were significantly high ($p < 0.01$ or $p < 0.05$) as compared to non-smoker at pretreatment, after the 3rd cycle and 6th cycle of chemotherapy but not well correlated with the PY exposures. Overall mean survival of smoker patients were significantly low when compared to non-smokers. The survival of > 50 PY group was significantly lowered ($p < 0.01$) as compared to others PY groups, indicating that survival after chemotherapy in smoker NSCLC patients may be dependent on their PY exposures. In conclusion, smoking is a bad prognostic factor in lung cancer therapy, besides its role in oxidative stress, and poor survival. Therefore, this factor can be used in patient selection for chemoprevention.**

Keywords: Lung cancer, smoking intensity, pack year, oxidative stress, prognostic factor

1. Introduction

Lung cancer is strongly associated with exposure to environmental carcinogens, with the highest risk being from cigarette smoking (*1*). Smoking is the most important risk factor for lung cancer, as supported by epidemiologic evidence since the 1950s (*2*,*3*). Thirty percent of all cancer is caused by smoking and approximately 85-90% of lung cancer cases are attributed to it (*4*,*5*).

Cigarette smoke (CS) can be divided into two phases; the gaseous phase and particulate matter (tar). Both phases are harmful, containing high concentration of toxic and carcinogenic compounds (*6*) and are both associated with diverse pulmonary disorders, including cancers. Although it is well established that tar contains a large number of carcinogens, previous studies suggest that chemicals in the gaseous phase of tobacco smoke are

**Address correspondence to:*
Dr. Anand N. Srivastava, Department of Pathology, Chhatrapati Shahuji Maharaj Medical University (Erstwhile King George's Medical University), Lucknow 226003(UP), India.
e-mail: inansrivastava@gmail.com; abhilashagupta02@gmail.com

of major importance in the cytotoxic and carcinogenic effects of tobacco on bronchopulmonary epithelial cells (*7,8*). At present it is well known that for these lesions to occur both phases of tobacco smoke are required (*9*).

Furthermore, cigarette smoke is a major public health hazard which exposes the respiratory tract to substantial oxidative stress. Benzo(a)pyrene, one of the most representative carcinogens of tobacco smoke, may be the origin of free radical derivatives (*10*). One puff from a cigarette contains approximately 10^{14} oxidant radicals in the combined gaseous and particulate states. Cigarette smoke contains over 4,700 chemical compounds, a high concentration of oxidants (10^{14} molecules/puff), and 3,000 ppm NO/puff (*11*). The nature of oxidant species found within CS varies from short lived oxidants, such as super oxide radical (O_2^-) and the nitric oxide molecule (NO), to long lived organic radicals, such as, semiquinones that can undergo redox cycling within the epithelial lining fluid of smokers for an extended amount of time (*12,13*).

It was also seen that formation of free radicals and consequent lipid peroxidation (LPO) has been associated with lung cancer (*14*). The principal radical in the tar phase, a quinine/hydroquinone complex, is capable of reducing molecular oxygen to superoxide radicals (*15*). The gas phase of CS contains small oxygen and carbon centered radicals that are much more reactive than are all tar phase radicals (*11*). The major antioxidant in lung lining fluid is reduced glutathione (GSH) (*16*). It can also be found intracellularly and at lower concentrations in plasma. It is a powerful scavenger of both reactive oxygen and nitrogen species and can protect proteins against nitration, particularly from the nitrogen dioxide radical, nitrous oxide found in cigarette smoke (*17*).

In addition, smoking is associated with factors that may contribute to poor cancer survival (*18*). There is now strong evidence that lung cancer in non-smokers shows different patterns than those observed in smokers (*19,20*). Several studies have also shown a positive association between smoking status and shortened survival after treatment (*21-24*).

Previous studies indicated that lower smoking intensity is associated with favorable overall survival of non-small cell cancer (*25,26*). This is because smoking affects the rates of metabolism for several drugs. It was also seen that lung functions in smokers may be more chemo resistant (*4*). The resistance of cancer cells to anticancer drugs is a serious clinical problem encountered in the chemotherapy (CT) of lung cancer patients.

We hypothesized that because smoking is a bad prognostic factor in lung cancer therapy, besides its role in oxidative stress and lung cancer genesis, that this factor can be used in patient selection for chemoprevention. The aim of the present work was to study the relationship of different smoking intensity with oxidative stress and survival after platinum based chemotherapy in non-small cell lung cancer patients.

2. Materials and Methods

2.1. *Patient characteristics*

Two hundred and three non-small cell lung cancer (NSCLC) patients (age, range 30-88 years) (155 males, 48 females) previously untreated, histologically or cytologically confirmed, admitted to the Department of Pulmonary Medicine, Chhatrapati Shahuji Maharaj Medical University, (C.S.M.M.U) Lucknow, India, and 144 age and sex matched healthy subjects (Control) were recruited for the study from October 2006 to December 2008. Eligible patients had an eastern cooperative oncology group (ECOG) performance status (PS) of 0, 1, 2, and 3. Chest radiographs and computed tomography for staging, sputum cytology, lavage examination, bronchoscopic biopsy, fine needle aspiration biopsy and cytology (if required) were performed in all lung cancer patients for histological diagnosis (adenocarcinoma, squamous cell carcinoma, large cell, and others), stage (IIIA, IIIB, and IV), and site of lesion.

Smoking data were recorded for all cases. All subjects were systematically interviewed through a standard questionnaire about their current and life time smoking status; detailed data were obtained about tobacco consumption, including: smoking start age, current smoking status, duration, intensity, amount of pack year of smoking, and time since quitting were noted. The patients were classified into two categories based on cigarette smoking status: non-smoker, *i.e*. patients who had never smoked or smoked less than 100 cigarettes in their life time; and smokers, *i.e*. patients who smoked and who continued smoking. The patients who had stopped smoking recently were considered as current smokers. Patients who had quit smoking for more than 1 year were considered former smokers. Smoking intensity of the smokers was calculated as pack year (PY) smoked. The total number of pack year was calculated by multiplying the number of packs smoked a day by the number of years of regular cigarette smoking. To explore whether smoking intensity at diagnosis is an independent prognostic factor, the smallest significant cutoff smoking intensity was identified by patients' stratification. Patients were stratified into 3 groups (1-20, 21-50, and > 50) by different smoking intensity cutoff values in the analysis. In our study the use of a cutoff point of PY was based on a previous study (*26*).

Patients received cisplatin (50-75 mg/m^2 of body surface area) divided into 3 doses on day 1, 2, and 3 and etoposide (70-100 mg/m^2 of body surface area) on day 1, 2 and 3 repeated every 3 weeks for a maximum of six cycles. Date of therapy initiation, date of therapy discontinuation, date of death, date of last follow-up, and status at last follow-up were recorded. The survival time was defined as the interval between the date of initial treatment and the date of last follow-

up examination. Patients who were deceased were calculated from the last date they were known to be alive based on the date of last contact. This date was verified by inpatient and outpatient medical records, and/or confirmation with the patient's primary care physician and/or family. Details of demographic characteristics of patients are given in Table 1.

The study protocol was approved by the ethical committee of Chhatrapati Shahuji Maharaj Medical University Lucknow, Uttar Pradesh, India (vide communication, *ref.* code- XXII ECM/P9). Before enrolment, written informed consent from each subject was obtained.

2.2. *Biochemical assay*

Blood specimens (5 mL) were aseptically drawn in EDTA prior to initiation of each chemotherapy course (first day). Blood samples were again collected after the 3rd and 6th cycle of chemotherapy for oxidative stress measurement. Levels of LPO, NO, GSH, and SOD were assessed at pretreatment (day '0') and post treatment (after 3rd and 6th cycle). Hemolysate was prepared using the method of Beutler *et al*. (*27*). The baseline (day '0') values of all these biochemical parameters were also assessed in 144 control subjects.

Total amount of lipid peroxidation products was estimated using the thiobarbituric acid (TBA) method, which measured the malondialdehyde (MDA)-TBA complex (*28*). The intensity of pink color of the product was read at 532 nm. Results were expressed as nmol MDA/mL. Activity of superoxide dismutase was determined by the method of McCord and Fridovich (*29*). The xanthine/xanthine oxidase system was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which was monitored at 560 nm. The level of this reduction was used as a measure of SOD activity. The reduced glutathione (GSH) level was determined using the method of Ellman *et al*. (*30*). The technique involved protein precipitation by metaphosphoric acid, and a spectrophotometric assay at 412 nm of the yellow derivative obtained from the reaction of supernatant with 5,5'-dithio-bis-2-nitrobenzoic acid. Plasma nitrite levels were measured with the method of Green *et al*. (*31*), using Griess reagent (sulfanilamide and *N*-(1-naphthyle)ethylenediamine). The method is based on a two step process. The first step is the conversion of nitrate to nitrite using nitrate reductase. The second step is the addition of Griess reagent that converts nitrite into a deep-purple azo compound. Photometric measurement of the absorbance at 540 nm of this azochromophore accurately determines the nitrite concentration (sodium nitrate is used as a standard) using a Microlab 300 semi-automated clinical chemistry analyzer (vital scientific) Merck, The Netherlands.

2.3. *Statistical analysis*

Independent groups were compared by using one way analysis of variance (ANOVA) while independent and dependent groups were compared with two factor repeated measures ANOVA followed by Newman-Keuls post hoc test. The Kaplan-Meier methods (Log rank test and Cox proportional hazard ratio) were used to compare survival between groups. A two-tailed ($\alpha = 2$), probability (p) value $p < 0.05$ was considered to be statistically significant. Graph Pad Prism (version 5) and STATISTICA (version 7) were used for the analysis.

For easy interpretation of the data, the percent mean change (from baseline to final evaluation) of one group over another was also evaluated as

$$\text{Mean change (\%)} = \frac{\text{Mean}_1 - \text{Mean}_2}{\text{Mean}_1} \times 100$$

where Mean_1 and Mean_2 denote means of 1st and 2nd groups, respectively.

Table 1. Demographic characteristics of patients

Characteristics	Number (%)
No. of patients	203 (100 %)
Sex - Male : Female	155 (76.4%): 48 (23.6%)
Smoker : Non-smoker	141 (69.5%): 62 (30.5%)
Pack year (PY) smoked , Mean ± SD, Median, Range	36 ± 26.1, 30, 3-162
PY-1-20 : PY-21-50 : PY- > 50 (n = 141)	36 (25.5%): 80 (56.8%): 25 (17.7%)
Age (Years)- Median, Range	55, 30-88
ECOG performance status- 0 : 1: 2	40 (19.7%): 107 (52.7%): 56 (27.6%)
Disease stage- IIIA : III B : IV	15 (7.4%): 142 (70.0%): 46 (22.7%)
Histological type- SCC: AC: LCC: O	82 (40.4%): 57 (28.1%): 27 (13.3%): 37 (18.2%)
Follow-up time (weeks)	88

Abbreviations: ECOG, Eastern co-operative oncology group performance status; SCC, squamous cell carcinoma; AC, Adenocarcinoma; LCC, large cell carcinoma; O, others (mixed).

3. Results

3.1. Oxidative stress levels of normal healthy subjects and non-smoker and smoker NSCLC patients at baseline

The baseline (pretreatment) oxidative stress levels of LPO, NO, GSH and SOD of normal healthy subjects (control) and non-smoker and smoker NSCLC patients (patients) are summarized in Table 2. Table 2 showed that pretreatment mean levels of LPO and NO in patients were comparatively high while the levels of GSH and SOD were comparatively low as compared to respective levels of control subjects and the increase and decrease were high in smokers as compared to non-smokers.

On comparing the mean level of LPO and NO in both non-smoker and smoker NSCLC patients were found to be significantly different and higher ($p < 0.01$) while the level of GSH and SOD were significantly lower ($p < 0.01$) as compared to control (Table 2). Similarly, the mean level of LPO and NO in NSCLC smoker patients were also found to be significantly higher ($p < 0.05$) while the level of GSH and SOD were significantly lower ($p < 0.05$) as compared to NSCLC non-smoker patients (Table 2).

Table 2. Baseline (pretreatment) biochemical parameters of normal healthy subjects (control) and NSCLC patients

Parameters	Control (n = 144)	NSCLC patients	
		Non-smoker (n = 62)	Smoker (n = 141)
LPO	2.24 ± 0.47	6.24 ± 0.92[a]	6.45 ± 0.70[a,b]
NO	11.88 ± 3.48	25.84 ± 4.58[a]	26.09 ± 5.58[a,b]
GSH	11.77 ± 1.30	3.20 ± 1.02[a]	3.01 ± 1.00[a,b]
SOD	2.89 ± 0.48	1.49 ± 0.61[a]	1.38 ± 0.56[a,b]

Data are shown as mean ± SD; [a] $p < 0.01$ (control *vs*. non-smoker or smoker); [b] $p < 0.05$ (non-smoker *vs*. smoker).

3.2. Oxidative stress levels of non-smokers and smokers NSCLC patients before and after chemotherapy

The oxidative stress levels of LPO, NO, GSH, and SOD in non-smoker and smoker NSCLC patients before (pretreatment or 0 cycles) and after 3rd and 6th cycles of chemotherapy are summarized in Table 3.

Comparing the levels within the groups (between cycles) (Table 3), the mean level of LPO and NO in both the non-smoker and smoker NSCLC patients at 3rd and 6th cycles were found to be significantly higher ($p < 0.01$) as compared to their respective pretreatment (0 cycle) levels. The respective levels of these in both the non-smoker and smoker NSCLC patients at 6th cycles were also found to be significantly higher ($p < 0.01$) as compared to their respective levels at 3rd cycle. However, the mean levels of GSH and SOD in both the non-smoker and smoker NSCLC patients at 3rd and 6th cycles were found to be significantly lower ($p < 0.01$) as compared to their respective pretreatment levels. The respective levels of these in both the non-smoker and smoker NSCLC patients at 6th cycles were also found to be significantly lower as compared to their respective levels at 3rd cycle.

Similarly, comparing the levels between the groups (non-smoker *vs*. smoker), the mean level of all oxidative stress parameters between the two groups at pretreatment were found to be the same, that is, levels did not differ significantly ($p > 0.05$) while at 3rd and 6th cycles these differed significantly (either $p < 0.05$, $p < 0.01$).

The percent mean change (0 cycles-6th cycles) (Table 3, last column) also showed more aggragation in smoker NSCLC patients than the non-smoker NSCLC patients. The LPO, NO, GSH, and SOD in NSCLC smoker patients aggragated (*i.e*. ratio of percent mean change of non-smoker and smoker) by a factor of 1.2, 1.2, 1.2, and 1.3 times more respectively than the non

Table 3. Biochemical parameters of non-smoker and smoker NSCLC patients before and after 3rd and 6th cycles of chemotherapy

Variables	Patients	At '0' cycle	After 3rd cycle	After 6th cycle	Mean change (%)
LPO	Non-smokers	6.24 ± 0.92 (62)	6.70 ± 0.92[a] (61)	7.22 ± 0.88[a,b] (53)	7.2
	Smokers	6.45 ± 0.70 (141)	7.04 ± 0.69[a] (131)	7.68 ± 0.60[a,b] (92)	8.3
NO	Non-smokers	25.84 ± 4.58 (62)	30.24 ± 4.25[a] (61)	31.83 ± 3.97[a,b] (53)	5.0
	Smokers	26.09 ± 5.58 (141)	32.01 ± 5.86[a] (131)	33.75 ± 4.53[a,b] (92)	5.2
GSH	Non-smokers	3.20 ± 1.02 (62)	2.53 ± 0.99[a] (61)	2.04 ± 0.92[a,b] (53)	7.2
	Smokers	3.01 ± 1.00 (141)	2.23 ± 0.86[a] (131)	1.70 ± 0.67[a,b] (92)	8.3
SOD	Non-smokers	1.49 ± 0.61 (62)	1.10 ± 0.55[a] (61)	0.98 ± 0.56[a,b] (53)	12.1
	Smokers	1.38 ± 0.56 (141)	0.95 ± 0.29[a] (131)	0.78 ± 0.20[a,b] (92)	23.0

Data are shown as mean ± SD. Values in parentheses are number of patients; [a] $p < 0.01$ (0 cycle *vs*. 3rd cycle or 6th cycle); [b] $p < 0.01$ (3rd cycle *vs*. 6th cycle).

smoker NSCLC patients. In other words, oxidative stress was well correlated with the patient exposures (non smoking and smoking).

3.3. *Pack year wise oxidative stress levels of smokers NSCLC patients before and after chemotherapy*

The pack year wise oxidative stress levels of LPO, NO, GSH, and SOD in smoker NSCLC patients before and after 3rd and 6th cycles of chemotherapy are summarized in Table 4.

Comparing levels within the groups (between cycles) (Table 4), the mean level of LPO and NO in all pack year groups of smoker NSCLC patients increased significantly ($p < 0.01$) after 3rd and 6th cycles of chemotherapy as compared to their respective pretreatment (0 cycle) levels. The respective levels of these were also significantly higher ($p < 0.001$) at 6th cycle as compared to their respective levels at 3rd cycle. Similarly, the mean levels of GSH and SOD in all pack year groups of smoker NSCLC patients decreased significantly ($p < 0.001$) after 3rd and 6th cycles of chemotherapy as compared to their respective pretreatment levels. The respective levels of these were also significantly lower ($p < 0.05$ or $p < 0.001$) at 6th cycle as compared to their respective levels at 3rd cycle.

Similarly, comparing the levels between the groups, except SOD, the mean level of all oxidative stress parameters in all three periods were found to be the same, that is, levels did not differ significantly ($p > 0.05$). The mean SOD of pack year > 50 at 0 cycle and 3rd cycle was found to be significantly higher ($p < 0.05$) than the pack year 1-20 and 21-50 groups.

The percent mean change (0 cycles-6th cycles) (Table 4, last column) of all oxidative stress parameters did not show any trend with pack year groups. In other words, oxidative stress did not correlate significantly with pack year exposures.

3.4. *Survivals*

The two years (or 88 wks) overall survival of all NSCLC patients (smoker + non-smoker), between non smoker and smoker NSCLC patients and among smoker NSCLC patients (pack year wise) has been summarized graphically in Figure 1.

The overall median survival of all NSCLC patients was found to be 28.50 wks (Figure 1a). The overall median survival of smoker NSCLC patients was significantly lower (Log rank test: $\chi^2 = 12.86$; $p < 0.01$) as compared to non-smoker NSCLC patients and the death rates in smoker NSCLC patients were also 0.22 times higher (Hazard ratio: ratio = 0.22; 95% CI of ratio = 0.19 to 0.61) than the death rate of non-smoker NSCLC patients (Figure 1b). Similarly, the overall median survival among different pack year groups of smoker NSCLC patients differed significantly (Log rank test: $\chi^2 = 27.53$; $p < 0.01$) with each other (Figure 1c). The survival of pack year > 50 smoker NSCLC patients was significantly lower as compared to the other pack year groups. Though the survival of pack year 21-50

Table 4. Pack year wise biochemical parameters of smoker NSCLC patients before and after 3rd and 6th cycles of chemotherapy

Parameters	Pack year	At '0' cycle	After 3rd cycle	After 6th cycle	Mean change (%)
LPO	1-20	6.44 ± 0.75 (36)	6.99 ± 0.75[a] (36)	7.82 ± 0.65[a,c] (27)	17.6
	21-50	6.46 ± 0.69 (80)	7.09 ± 0.64[a] (74)	7.67 ± 0.51[a,c] (58)	15.8
	> 50	6.46 ± 0.68 (25)	6.94 ± 0.79[a] (21)	7.25 ± 0.89[a,d] (7)	11.0
NO	1-20	25.88 ± 6.31 (36)	31.61 ± 6.35[a] (36)	34.39 ± 5.42[a,c] (27)	24.8
	21-50	26.28 ± 5.57 (80)	31.63 ± 5.80[a] (74)	33.25 ± 4.21[a,c] (58)	21.0
	> 50	25.76 ± 4.57 (25)	34.04 ± 4.96[a] (21)	35.49 ± 2.82[a,d] (7)	27.4
GSH	1-20	3.11 ± 1.07 (36)	2.20 ± 0.90[a] (36)	1.60 ± 0.65[a,c] (27)	48.4
	21-50	2.96 ± 0.97 (80)	2.31 ± 0.85[a] (74)	1.73 ± 0.68[a,c] (58)	41.5
	> 50	3.03 ± 1.04 (25)	1.99 ± 0.80[a] (21)	1.84 ± 0.68[b,d] (7)	39.1
SOD	1-20	1.32 ± 0.47 (36)	0.93 ± 0.24[a] (36)	0.81 ± 0.21[a,c] (27)	39.1
	21-50	1.30 ± 0.53 (80)	0.92 ± 0.24[a] (74)	0.76 ± 0.18[a,c] (58)	41.5
	> 50	1.73 ± 0.63 (25)	1.13 ± 0.43[a] (21)	0.79 ± 0.37[a,c] (7)	54.0

Data are shown as mean ± SD. Values in parentheses are number of patients; [a] $p < 0.01$ (0 cycle *vs*. 3rd cycle or 6th cycle); [b] $p < 0.05$ (0 cycle *vs*. 6th cycle); [c] $p < 0.01$ (3rd cycle *vs*. 6th cycle); [d] $p < 0.05$ (3rd cycle *vs*. 6th cycle).

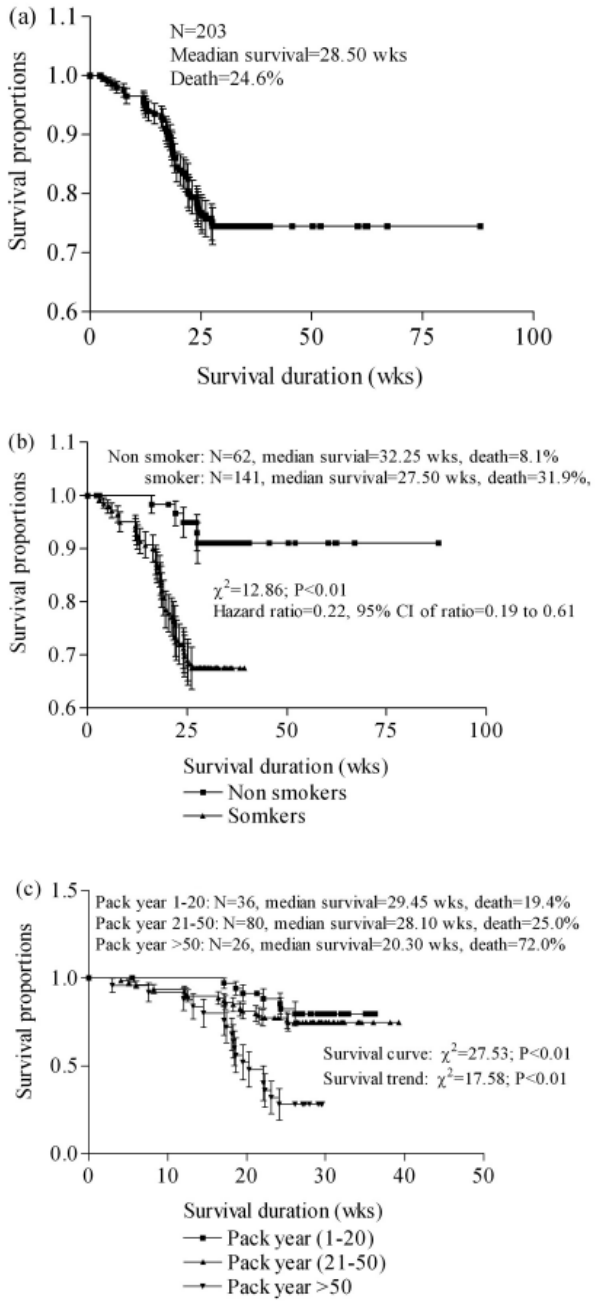

Figure 1. The two years overall survival. (a), All NSCLC patients; **(b)**, Non-smoker and smoker NSCLC patients; **(c)**, Different pack year groups of smoker NSCLC patients.

smoker NSCLC patients was also lower as compared to pack year 1-20 but seems statistically insignificant ($p > 0.05$) (Figure 1c). The survival trend among the three pack year exposure groups was also found to be significant (Log rank test for trend: $\chi^2 = 17.58$; $p < 0.01$) indicating that survival after chemotherapy in smoker NSCLC patients may be dependent on their pack year exposure.

4. Discussion

Cigarette smoking is a well known environmental risk factor for lung carcinogenesis. Tobacco smoke contains many mutagenic and carcinogenic chemicals (*32*,*33*) that might be associated with mutation in genes (*34*,*35*). Cigarette smoke is a composite of numerous pollutants in rather high concentrations. Well over one thousand constituents of smoke, including many oxidants, pro-oxidants, free radicals and reducing agents, have been identified (*36*). Also, Pryor and associates (*11*,*15*) have identified two different populations of free radicals , one in tar and the other in gas phase of CS. The principal radical in the tar phase, a quinone/hydroquinone complex is capable of reducing molecular oxygen to superoxide radicals. The gas phase of cigarette smoke contains small oxygen and carbon centered radicals that are much more reactive than are all tar phase radicals. Thus cigarette smoke contains many oxidants, free radicals and metastable products derived from free radical reactions that are capable of reacting with or inactivating essential cellular constituents.

An increased oxidant burden in smokers derives from the fact that cigarette smoke contains an estimated 10^{14} oxidants and 3,000 ppm NO/puff, and many of these are relatively long lived including tar-semiquinone, which can generate OH^- and hydrogen peroxide in the presence of free iron through the Fenton reaction (*11*). The increased oxidative metabolism of phagocytes is accompanied by increased generation of reactive oxygen species (ROS); such as, hydrogen peroxide, hydroxyl radicals and superoxide radicals. These ROS can attack DNA directly or cause membrane damage, and they can activate oxygen, a process that has been associated with tumor promotion. Earlier studies found that smokers have higher plasma levels of lipid peroxidation products, measured through the thiobarbituric acid (TBA)-malondialdehyde method (*37*). We therefore studied lipid peroxidation in smoker and non-smoker lung cancer patients. In addition there is a decrease in antioxidants in the distal airways of smokers, as well as a decrease in vitamin E in the bronchoalveolar lavage fluid, when compared to non smokers (*38-40*).

Some studies also discussed that CS increases the formation of reactive nitrogen species (RNS) and results in nitration and oxidation of plasma proteins. The levels of nitrated proteins (fibrinogen, transferrin, plasminogen, and ceruloplasmin) were higher in smokers compared to non-smokers (*41*). Evidence of increased $NO/ONOO^-$ activity in plasma and epithelial lining fluid has been shown in chronic smokers resulting in elevated formation of 3-nitrotyrosine (*42*).

The major antioxidants in lung lining fluid are GSH (*16*). Cofgreave *et al*. have shown that acute cigarette smoke inhalation for one hour caused significant depletion of GSH in the lungs, lavaged cells and lavaged fluid of rats (*43*). GSH is a powerful scavenger of both ROS and RNS and can protect proteins against nitration, particularly from the nitrogen dioxide radical, and nitrous oxide found in cigarette smoke (*17*).

The actual mechanisms of the carcinogens have

not been identified, even though polycyclic aromatic hydrocarbon and tobacco specific nitrate compounds have been indicated (*44*). Cigarette smoke alters the lung metabolism of many endogenous compounds as well as the activities of many biotransforming enzymes in lung tissue (*45*). It has been also well established that smoking is associated with many mortality-related pathological conditions and causes irreversible damage to lung parenchyma (*46*).

Smoking also affects the rates of metabolism for several drugs (*4*). In addition, it is possible that lung tumors in smokers may be more chemo resistant. The actual mechanism of this drug resistance is under investigation (*4*). The resistance of cancer cells to anticancer drugs is a serious clinical problem encountered in the chemotherapy of lung cancer patients.

In addition, smoking is associated with factors that may contribute to poor cancer survival; an increased mutation burden that could lead to accelerated carcinogenesis and progression (*47*). This is one of the most important determinants because smoking is associated with numerous diseases (*18,48*). Thus patients with lung cancer with a smoking history are at risk of dying from a spectrum of smoking associated diseases.

Despite the indisputable link between smoking status and increased risk of lung cancer, the data on the inclusion of this predictor in prognostic survival analysis has been scarce. Although a large number of papers have been published evaluating prognostic factors in lung cancer, tumor node metastasis (TNM)-staging is the most important tool to estimate the prognosis of lung cancer patients and to define the best treatment modality (*49*). TNM-staging gives an accurate estimate of localization and disease progression at the time of diagnosis, but it does not account for survival differences within the same stage. Pretreatment variables on survival are not necessarily identical to predicting response to chemotherapy (CT). Therefore, identification of these factors would be useful in analysis of the response rate to CT in lung cancer patients. Recently, a study indicated a beneficial effect on ECOG performance status when patients stopped smoking after diagnosis of NSCLC (*50*). Previous studies (*51,52*) suggest that the benefit consistently increases with the number of years since smoking abstinence. This is important since the prognosis of lung cancer patients might significantly be improved by smoking cessation (*53*). Current smoking at the time of diagnosis was an independent predictor of poor prognosis in lung cancer patients (*54*). Furthermore, a worse survival rate was seen in 215 SCLC patients who smoked throughout chemotherapy (*55*). However, a study with 154 SCLC patients showed that continued smoking during chemotherapy did not affect the outcome of this treatment (*24*). Our study examined whether smoking intensity by inducing oxidative stress affected the survival of patients with lung cancer treated with chemotherapy.

We observed a poorer response in patients with PY > 50. Patients with PY > 50 had a worse response than those with PY < 50. Factors related to tobacco smoking have been implicated in poorer response for current and former smokers. At least 55 of the > 2,000 chemical compounds identified in the tobacco leaf are proven carcinogens (*56*) and many of them could be responsible for the poor response. In a study of 369 patients with NSCLC, smoking pack years before surgery had a prognostic value (*46*). Also patients were classified into two group in a previous study with > 30 and < 30 smoking pack year. Smoking with 30 or more pack year was associated with poor prognosis and non-smokers have better prognosis compared with smokers (*25*). In a Japanese population based study stage I lung adenocarcinoma patients with a smoking intensity of less than 20 pack year showed a more favorable prognosis than those with a PY of 20 or more (*26*). It was seen that in our study that smokers show more oxidative stress than non-smokers before and after chemotherapy. Survival of the smoker NSCLC patients was also low as compared to non-smokers. Also the patients with > 50 PY showed worse survival than the other PY groups. We can conclude that smoking is a bad prognostic factor in lung cancer therapy, besides its role in oxidative stress, lung cancer genesis and poor survival. Therefore, this factor can be used in patient selection for chemoprevention.

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References

1. Bach PB, Kattan MW, Thornquist MD, Kris MG, Tate RC, Barnett MJ, Hsieh LJ, Begg CB. Variations in lung cancer risk among smokers. J Natl Cancer Inst. 2003; 95:470-478.
2. Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: a study of 684 proved cases. J Am Med Assoc. 1950; 143:329-336.
3. Doll R, Hill AB. A study of the aetiology of carcinoma of the lung. Br Med J. 1952; 2:1271-1286.
4. Dresler CM. Is it more important to quit smoking than which chemotherapy is used? Lung Cancer. 2003; 39:119-124.
5. Barlesi F, Nanni-Metellus I, Breen D, Fina F, Astoul P, Martin PM. Non-small cell lung cancer-smokers or non-smokers, does it matter? Lung Cancer. 2009; 63:430-432.
6. Hebert JR, Kabat GC. Distribution of smoking and its

association with lung cancer: implications for studies on the association of fat with cancer. J Natl Cancer Inst. 1991; 83:872-874.
7. Sonnenfeld G, Griffith RB, Hudgens RW. The effect of smoke generation and manipulation variables on the cytotoxicity of mainstream and sidestream cigarette smoke to monolayer cultures of L-929 cells. Arch Toxicol. 1985; 58:120-122.
8. Pouli AE, Hatzinikolaou DG, Piperi C, Stavridou A, Psallidopoulos MC, Stavrides JC. The cytotoxic effect of volatile organic compounds of the gas phase of cigarette smoke on lung epithelial cells. Free Radic Biol Med. 2003; 34:345-355.
9. Coggins CR, Fouillet XL, Lam R, Morgan KT. Cigarette smoke induced pathology of the rat respiratory tract: a comparison of the effects of the particulate and vapour phases. Toxicology. 1980; 16:83-101.
10. Sullivan PD. Free radicals of benzo(a)pyrene and derivatives. Environ Health Perspect. 1985; 64:283-295.
11. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect. 1985; 64:111-126.
12. Nakayama T, Church DF, Pryor WA. Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. Free Radic Biol Med. 1989; 7:9-15.
13. Zang LY, Stone K, Pryor WA. Detection of free radicals in aqueous extracts of cigarette tar by electron spin resonance. Free Radic Biol Med. 1995; 19:161-167.
14. Cerutti PA. Prooxidant states and tumor promotion. Science. 1985; 227:375-381.
15. Pryor WA, Hales BJ, Premovic PI, Church DF. The radicals in cigarette tar: their nature and suggested physiological implications. Science. 1983; 220:425-427.
16. Cross CE, van der Vliet A, O'Neill CA, Louie S, Halliwell B. Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect. 1994; 102 (Suppl 10):185-191.
17. Janoff A, Pryor WA, Bengali ZH. NHLBI workshop summary. Effects of tobacco smoke components on cellular and biochemical processes in the lung. Am Rev Respir Dis. 1987; 136:1058-1064.
18. Tammemagi CM, Neslund-Dudas C, Simoff M, Kvale P. Smoking and lung cancer survival: the role of comorbidity and treatment. Chest. 2004; 125:27-37.
19. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers--a different disease. Nat Rev Cancer. 2007; 7:778-790.
20. Toh CK. The changing epidemiology of lung cancer. Methods Mol Biol. 2009; 472:397-411.
21. Toh CK, Wong EH, Lim WT, Leong SS, Fong KW, Wee J, Tan EH. The impact of smoking status on the behavior and survival outcome of patients with advanced non-small cell lung cancer: a retrospective analysis. Chest. 2004; 126:1750-1756.
22. Nordquist LT, Simon GR, Cantor A, Alberts WM, Bepler G. Improved survival in never-smokers *vs* current smokers with primary adenocarcinoma of the lung. Chest. 2004; 126:347-351.
23. Fox JL, Rosenzweig KE, Ostroff JS. The effect of smoking status on survival following radiation therapy for non-small cell lung cancer. Lung Cancer. 2004; 44:287-293.
24. Bergman B, Sorenson S. Smoking and effect of chemotherapy in small cell lung cancer. Eur Respir J. 1988; 1:932-937.
25. Tan YK, Wee TC, Koh WP, Wang YT, Eng P, Tan WC, Seow A. Survival among Chinese women with lung cancer in Singapore: a comparison by stage, histology and smoking status. Lung Cancer. 2003; 40:237-246.
26. Yoshino I, Kawano D, Oba T, Yamazaki K, Kometani T, Maehara Y. Smoking status as a prognostic factor in patients with stage I pulmonary adenocarcinoma. Ann Thorac Surg. 2006; 81:1189-1193.
27. Beutler E, West C, Blume KG. The removal of leukocytes and platelets from whole blood. J Lab Clin Med. 1976; 88:328-333.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351-358.
29. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. J Biol Chem. 1969; 244:6056-6063.
30. Evans JC, Ellman GL. The ionization of cysteine. Biochim Biophys Acta. 1959; 33:574-576.
31. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982; 126:131-138.
32. Hackshaw AK, Law MR, Wald NJ. The accumulated evidence on lung cancer and environmental tobacco smoke. BMJ. 1997; 315:980-988.
33. Stellman SD, Resnicow K. Tobacco smoking, cancer and social class. IARC Sci Publ. 1997: 229-250.
34. Hung RJ, McKay JD, Gaborieau V, *et al*. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature. 2008; 452:633-637.
35. Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science. 1996; 274:430-432.
36. Schumacher JN, Green CR, Best FW, Newell MP. Smoke composition. An extensive investigation of the water-soluble portion of cigarette smoke. J Agric Food Chem. 1977; 25:310-320.
37. Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med. 1996; 154:1055-1060.
38. Theron AJ, Richards GA, Van Rensburg AJ, Van der Merwe CA, Anderson R. Investigation of the role of phagocytes and anti-oxidant nutrients in oxidant stress mediated by cigarette smoke. Int J Vitam Nutr Res. 1990; 60:261-266.
39. Hemila H, Roberts P, Wikstrom M. Activated polymorphonuclear leucocytes consume vitamin C. FEBS Lett. 1984; 178:25-30.
40. Chung-man Ho J, Zheng S, Comhair SA, Farver C, Erzurum SC. Differential expression of manganese superoxide dismutase and catalase in lung cancer. Cancer Res. 2001; 61:8578-8585.
41. Pignatelli B, Li CQ, Boffetta P, Chen Q, Ahrens W, Nyberg F, Mukeria A, Bruske-Hohlfeld I, Fortes C, Constantinescu V, Ischiropoulos H, Ohshima H. Nitrated and oxidized plasma proteins in smokers and lung cancer patients. Cancer Res. 2001; 61:778-784.
42. Petruzzelli S, Puntoni R, Mimotti P, Pulera N, Baliva F, Fornai E, Giuntini C. Plasma 3-nitrotyrosine in cigarette smokers. Am J Respir Crit Care Med. 1997; 156:1902-1907.

43. Cotgreave IA, Johansson U, Moldeus P, Brattsand R. The effect of acute cigarette smoke inhalation on pulmonary and systemic cysteine and glutathione redox states in the rat. Toxicology. 1987; 45:203-212.
44. Hoffmann D, Wynder EL. A study of tobacco carcinogenesis. XI. Tumor initiators, tumor accelerators, and tumor promoting activity of condensate fractions. Cancer. 1971; 27:848-864.
45. Petruzzelli S, Camus AM, Carrozzi L, *et al*. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. Cancer Res. 1988; 48:4695-4700.
46. Fujisawa T, Iizasa T, Saitoh Y, Sekine Y, Motohashi S, Yasukawa T, Shibuya K, Hiroshima K, Ohwada H. Smoking before surgery predicts poor long-term survival in patients with stage I non-small-cell lung carcinomas. J Clin Oncol. 1999; 17:2086-2091.
47. Sanchez-Cespedes M, Ahrendt SA, Piantadosi S, Rosell R, Monzo M, Wu L, Westra WH, Yang SC, Jen J, Sidransky D. Chromosomal alterations in lung adenocarcinoma from smokers and nonsmokers. Cancer Res. 2001; 61:1309-1313.
48. Miyazaki K, Satoh H, Kagohashi K, Kurishima K, Ishikawa H, Ohtsuka M, Sekizawa K. Lung cancer patients with previous or simultaneous urologic cancers. Med Oncol. 2008; 25:443-446.
49. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest. 1997; 111:1710-1717.
50. Baser S, Shannon VR, Eapen GA, Jimenez CA, Onn A, Lin E, Morice RC. Smoking cessation after diagnosis of lung cancer is associated with a beneficial effect on performance status. Chest. 2006; 130:1784-1790.
51. Ebbert JO, Williams BA, Sun Z, Aubry MC, Wampfler JA, Garces YI, Meyer RL, Yang P. Duration of smoking abstinence as a predictor for non-small-cell lung cancer survival in women. Lung Cancer. 2005; 47:165-172.
52. Zhang B, Ferrence R, Cohen J, Bondy S, Ashley MJ, Rehm J, Jain M, Rohan T, Miller A. Smoking cessation and lung cancer mortality in a cohort of middle-aged Canadian women. Ann Epidemiol. 2005; 15:302-309.
53. Dresler CM, Bailey M, Roper CR, Patterson GA, Cooper JD. Smoking cessation and lung cancer resection. Chest. 1996; 110:1199-1202.
54. Duarte RL, Luiz RR, Paschoal ME. The cigarette burden (measured by the number of pack-years smoked) negatively impacts the response rate to platinum-based chemotherapy in lung cancer patients. Lung Cancer. 2008; 61:244-254.
55. Videtic GM, Stitt LW, Dar AR, Kocha WI, Tomiak AT, Truong PT, Vincent MD, Yu EW. Continued cigarette smoking by patients receiving concurrent chemoradiotherapy for limited-stage small-cell lung cancer is associated with decreased survival. J Clin Oncol. 2003; 21:1544-1549.
56. Kuper H, Boffetta P, Adami HO. Tobacco use and cancer causation: association by tumour type. J Intern Med. 2002; 252:206-224.

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