RESEARCH COMMUNICATION

Cisplatin Combination Chemotherapy Induces Oxidative Stress in Advance Non Small Cell Lung Cancer Patients

Anand Narain Srivastava[1]*, Abhilasha Gupta[2]*, Shruti Srivastava[2], SM Natu[2], Balraj Mittal[3], MPS Negi[4], Rajendra Prasad[5]

Abstract

  <u>Background</u>: This study determine oxidative stress and survival prospectively in advanced stage non small cell lung cancer (NSCLC) patients following cisplatin based combination chemotherapy. <u>Materials and Methods</u>: The oxidative stress levels (LPO, NO, GSH and SOD) of 144 control subjects and 203 advanced stage (IIIA/IIIB/IV) newly diagnosed NSCLC patients were assessed at pre-treatment (day '0'), and after the 3rd and 6th cycles of chemotherapy. Groups were compared by repeated measures ANOVA while comparison of survival curves was conduced by Kaplan-Meier methods. <u>Results</u>: The pre-treatment mean levels of LPO and NO in patients were significantly ($P<0.01$) higher while GSH and SOD were significantly ($P<0.01$) lower as compared to control. The oxidative stress was elevated more significantly ($P<0.01$) after the chemotherapy and was more evident in higher stage than lower stage patients. The two year overall survival (%) of stage IV patients was significantly lower ($P<0.05$) as compared to stage III A and III B. The proportional mortality was also maximal in stage IV patients (37.0%) followed by stage III B (31.7%) and III A (20.0%). <u>Conclusion</u>: Cisplatin based combination chemotherapy induces oxidative stress in NSCLC patients.

Keywords: Chemotherapy - oxidative stress - stages - survival - non small cell lung cancer (NSCLC) patients

Introduction

Lung cancer is the leading cause of cancer mortality worldwide among both men and women (Parkin et al., 2005). Non small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases. Lung cancer staging using tumor-node-metastasis (TNM) system has undergone modifications since 1970 (Mountain, 1997). Tumors restricted to the lung in stage I and stage II should be removed surgically. Most patients with NSCLC are diagnosed in advanced stages III or IV disease at presentation (Jemal et al., 2007). The overall 5 year survival for NSCLC remains poor (15%); however if the cancer is detected at stage IA, the 5 year survival often exceeds 80% and stage IIIA, IIIB, and IV remains 13%, 7% and 1% respectively (Mulshine and Sullivan, 2005).

Prognostic factors are essential in the management of patients with lung cancer (Kumar et al., 2009). It is well known that survival is strongly related to the performance status and to the stage of the disease at diagnosis in lung cancer patients (Albain et al., 1991). In accordance with the literature, the comparison among the survival curves for each clinical stage revealed more aggressive behaviour and more rapidly progressing disease in more advanced stages (Novaes et al., 2008). Most patients with a diagnosis of lung cancer are treated with cisplatin based combination chemotherapy and the response is related to tumor and clinical characteristics, such as stage and performance status, as well as to drug, sensitivity of the host and tumor tissues (Relling and Dervieux, 2001).

Of the most active and widely used anticancer drugs, cisplatin is known to generate reactive oxygen species (ROS) (Weijl et al., 1997). These highly ROS can cause extensive tissue damage through reaction with all biological macromolecules e.g. lipids, proteins and nucleic acids, leading to the formation of oxidized substances such as the membrane lipid peroxidation (LPO) product malondialdehyde (MDA) (Sangeetha et al., 1990; Look and Musch, 1994). To circumvent the damages caused by the ROS, multiple defence systems, collectively called antioxidants; superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione (GSH) etc. are present in human serum as well as erythrocytes (Patterson and Leake, 1998; Kaynar et al., 2005). It was demonstrated that plasma concentration of various antioxidants decreased significantly during cisplatin based chemotherapy in cancer patients (Weijl et al., 1998) resulting oxidative stress. The high level of

[1]*Department of Pathology, Era's Lucknow Medical College & Hospital, Sarfarazganj, Moosa bagh, Picnic spot,* [2]*Department of Pathology,* [3]*Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences,* [4]*Division of Biometry and Statistics, Central Drug Research Institute,* [5]*Department of Pulmonary Medicine, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India *For Correspondence : inansrivastava@gmail.com, abhilashagupta02@gmail.com*

oxidative stress during chemotherapy may overcome the antioxidant defence of cancer cells, halting cancer cell proliferation and interfering with antineoplastic activity (Esme et al., 2008). Thus the antioxidant status of cancer patients may play an important role in their response to chemotherapy (Nowak and Janczak, 2006).

As survival of the patients is strongly related to the stage of disease in NSCLC, therefore this study was aimed to investigate oxidative stress status in different stages of NSCLC patients and their response and survival after the cisplatin based combination chemotherapy.

Materials and Methods

Two hundred and three NSCLC patients (age, range 30-88 years) (155 males, 48 females) previously untreated, histologically or cytologically confirmed and admitted in the Department of Pulmonary medicine, of the related institute, and 144 age and sex matched healthy subjects (Controls) 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 (CT) 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. Histology (adenocarcinoma, squamous cell carcinoma, large cell, and others), stage (IIIA, IIIB and IV), side of lesion, date of therapy initiation, date of therapy discontinuation, date of death, date of last follow-up, status at last follow-up was 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 not diseased were censored at 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 the related institute (vide communication, ref. code- XXII ECM/P9). Before enrolment, written informed consent from each subject was obtained.

Patients received cisplatin (50-75 mg/m^2 of body surface area) divided in to 3 doses on day 1, 2 and 3 and etoposide (70-100 mg/m^2 of body surface area) on 1, 2 and 3 days repeated every 3 weeks for a maximum six cycles.

Blood specimens (5ml) were aseptically drawn in EDTA prior to initiation of each chemotherapy course (first day). Blood was also taken after 3rd and after 6th cycle of the chemotherapy for oxidative stress measurement. Erythrocytes were separated from whole blood (Beutler et al., 1976). EDTA blood was centrifuged at 3000 rpm for 15 minutes in cold centrifuge at 4ºC, Plasma was carefully separated. Erythrocytes were washed with chilled normal saline (0.85%NaCl) three times, haemolysed by adding chilled water, and haemolysate made up to original volume followed by freezing and thawing. After centrifugation at 3000 rpm for 15 minutes at 4ºC in cold at 3000 rpm for 15 minutes, precipitate was removed and cleared supernatant was collected for antioxidants activity. Protein was estimated in haemolysate (Lowry et al., 1951).

Total amount of lipid peroxidation products was estimated using thiobarbituric acid (TBA) (Ohkawa et al., 1979), which measured the MDA-TBA complex. For this method 200 μl of blood plasma was mixed with 0.5 ml glacial acetic acid. Subsequently, 0.5 ml of 8% sodium dodecyl sulphate was added to the above reaction mixture. After mixing well 1.5 ml of 0.8% TBA solution was added. The reaction mixture was kept in boiling water bath for 1 hour. After cooling to room temperature 3.0 ml of n-butanol was mixed, the reaction mixture was then centrifuged at 10,000 ×g for 15 min. The intensity of the pink colour of the obtained fraction product was read at 532 nm. Results were expressed as nm MDA /ml.

Two reaction setups were run in parallel for Superoxide Dismutase (SOD) estimation (McCord and Fridovich, 1969). The tubes in the first setup (experimental) received 0.2 ml (320μM) nitroblue tetra-zolium (NBT), 0.2 ml (10μM) pnenazine methosulfate, 2.0ml (0.16 mM) pyro-phosphate buffer pH 9.2, 0.02 of plasma as enzyme source. The tubes in the second setup (reference) received the entire above reagents except the enzyme source. The reaction was started simultaneously in both sets by the addition of 0.2 ml (160 μM) of NADH. After an interval of 90 seconds, 1 ml of glacial acidic acid was added to each reaction tube. The reference tubes were then added with the same amount of enzyme source, and absorbance was read at 560 nm against a blank on spectrophotometer.

The reduced glutathione level was determine in plasma (Evans and Ellman, 1959). For this 100 μl of plasma was mixed with 3.0 ml of 5% (w/v) tri chloro acetic acid (TCA) reagent and allowed to stand for 5 minutes; proteins were precipitated and filtered out. Later, 2.0 ml of filtrate was added to 4.0 ml of 0.3 M phosphate buffer pH 7.4 and 1 ml of 5-5' dithio-bis-2 nitrobenzoic acid (DTNB) (1% w/v aqueous sodium citrate). A blank was prepared in a similar manner using distilled water in place of the filtrate. An appropriate standard solution of 0.1 ml GSH (10 μmol) was also run simultaneous. The pale yellow colour developed was read immediately at 412 nm by spectrophotometer.

Table 1. Demographic Characteristics of Patients

Characteristics	Number(%)
No. of patients	203(100%)
Sex- Male:Female	155(76.4%): 48(23.6%)
Age (yrs)	55, 30-88
- Median, range	
*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 (wks)	88

*ECOG-Eastern Co-operative Oncology Group performance status, *SCC-Squamous Cell Carcinoma, *AC-Adenocarcinoma, *LCC-Large Cell Carcinoma, *O-Others(Mixed)

Plasma nitrite levels were measured (Green et al., 1982) with the use of Griess reagent {sulfanilamide and N-(1-naphthyle) ethylenediamine}. The method is based on 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, which converts nitrite into a deep-purple azo compound photometric measurement of the absorbance at 540 nm due to this azochromophore accurately determines the nitrite concentration (sodium nitrate is used as standard). Protein interference was eliminated by treatment of the reacted samples with zink sulfate and centrifugation for 5 min at 10,000 g. *(MERCK, Microlab 300, Semi automated clinical chemistry analyser, Vital scientific; The Netherlands).*

Statistical analysis

The independent groups were compared by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test and dependent groups by repeated measures ANOVA followed by Tukey's post hoc test. The dependent and independent groups were also compared respectively by two sample paired and unpaired t-test. The Kaplan-Meier methods (Log rank test and Cox proportional hazard ratio) were used to compare the survival between the 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 interpretations of the data, the percent change between two groups/variables was evaluated as

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

where,

$\qquad$ Mean$_1$: Mean of Ist group/variable
$\qquad$ Mean$_2$: Mean of IInd group/variable

Results

The biochemical parameters of normal healthy subjects (control) and non small cell lung cancer patients (patients) are summarized in Table 2 and also shown graphically by Figure 1. Both Table 2 and Figure 1 showed that the base line ('0' cycle) mean values of LPO and NO in patients were higher while GSH and SOD were lower as compared to respective level of control subjects. After treatment, the mean levels of LPO and NO in patients increases linearly with increasing cycles of chemotherapy while GSH and SOD decreases. On comparing, the mean levels of LPO, NO, GSH and SOD in patients at all three cycles (periods) were found to be significantly (P<0.01) different than respective level of control subjects (Table 2 and Figure 1). The significant increase (% mean change wirh respect to control) in LPO and NO in patients at 0 cycle, 3rd cycles and 6th cycles were found to be 64.6%, 67.8% and 69.9% and 53.9%, 60.0% and 63.8% respectively while GSH and SOD decreased by 64.1%, 74.2% and 77.9% and 46.4%, 59.3% and 66.3% respectively.

The stage wise biochemical parameters of patients at

Table 2. Biochemical Parameters Summary (Mean ± SD) of Normal Healthy Subjects (Controls) and Non Small Cell Lung Cancer Patients (Patients)

Variables	Controls	Patients		
	(n=144)	0 cycle (n=203)	3rd cycle (n=192)	6th cycle (n=155)
LPO	2.24±0.47	6.32±0.70**	6.96±0.67**	7.43±0.67**
NO	11.88±3.48	25.76±4.54**	29.69±4.51**	32.81±4.27**
GSH	11.77±1.30	4.23±0.92**	3.04±0.88**	2.60±0.83**
SOD	2.89±0.48	1.55±0.56**	1.17±0.54**	0.97±0.46**

**P<0.01; Asterisk in superscript showed comparison of controls group with patients groups (cycle) and were significant at P<0.01
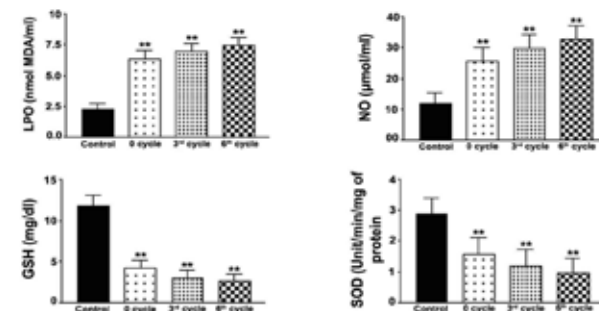

Figure 1. Bar Graph showing Average (± SD) Levels of LPO, NO, GSH, and SOD, in Normal Healthy Subjects (Control) and NSCLC (Patients). The levels of controls subjects were compared with patients and were significant at P<0.01, **P<0.01
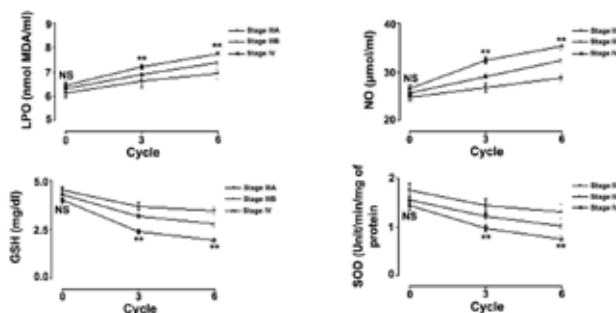

Figure 2. Line Graphs showing Stage Wise Average (± SE) Levels of LPO, NO, GSH and SOD in Patients with NSCLC. For each cycle, the level of biochemical parameter were compared between the stages; ns- P>0.05, **P<0.01.

0 cycle, 3rd cycles and 6th cycles of chemotherapy were further summarized in Table 3 and also shown graphically by Figure 2. Table 3 and Figure 2 both showed that the base line mean value of LPO and NO in patients increases with increasing stages while GSH and SOD decreases. Comparing the levels of each parameter between the stages, the baseline mean levels of all the parameters in all the three stages were found to be the same (P>0.05) i.e. not differed significantly (Figure 2). Similarly, the mean levels of LPO, NO, GSH and SOD in patients of stage IIIA and IIIB at 3rd and 6th cycles were found to be the same (P>0.05) while the levels of all these in patients of stage IV at both 3rd cycles and 6th cycles were found to be significantly (P<0.01) different than patients of stage IIIA and IIIB (Figure 2). Similarly comparing the levels of each parameter within the stages, the levels of LPO, NO,

Table 3. Stage Wise Biochemical Parameters Summary (Mean±SD) of Patients with Non Small Cell Lung Cancer

Variables	Stages	0cycle	3rd cycle	6th cycle	% change (from 0 cycle to 6th cycle)
LPO	Stage III A	6.16±0.83 (15)	6.63±0.97[a] (15)	6.93±0.75[ab] (13)	11.2%
	Stage III B	6.30±0.60 (142)	6.90±0.65[a] (132)	7.37±0.67[ab] (103)	14.6%
	Stage IV	6.44±0.92 (46)	7.22±0.54[a] (45)	7.76±0.49[ab] (39)	17.1%
NO	Stage III A	24.78±2.98 (15)	26.79±3.42[a] (15)	28.74±2.52[ab] (13)	13.8%
	Stage III B	25.63±4.60 (142)	29.09±4.17[a] (132)	32.34±3.87[ab] (103)	20.7%
	Stage IV	26.48±4.75 (46)	32.45±4.58[a] (45)	35.40±4.33[ab] (39)	25.2%
GSH	Stage III A	4.51±0.62 (15)	3.68±0.79[a] (15)	3.43±0.63[ab] (13)	24.0%
	Stage III B	4.28±0.87 (142)	3.19±0.81[a] (132)	2.75±0.76[ab] (103)	35.6%
	Stage IV	3.98±1.08 (46)	2.37±0.73[a] (45)	1.93±0.61[ab] (39)	51.5%
SOD	Stage III A	1.75±0.53 (15)	1.43±0.55[a] (15)	1.31±0.52[ab] (13)	25.2%
	Stage III B	1.56±0.56 (142)	1.22±0.54[a] (132)	1.01±0.46[ab] (103)	35.0%
	Stage IV	1.44±0.57 (46)	0.96±0.47[a] (45)	0.75±0.33[ab] (39)	48.0%

The superscript 'a' compares mean level at 0 cycle with 3rd cycle and 6th cycle and were significant at $P<0.01$. Similarly, superscript 'b' compares mean level at 3rd cycle with 6th cycle and were significant at $P<0.01$.

GSH and SOD in patients of all three stage at 3rd cycles and 6th cycles were found to be significantly ($P<0.01$) different than respective levels at 0 cycle while the levels of all these in all three stages at 6th cycles were also found to be significantly ($P<0.01$) different than the respective levels at 3rd cycles (Table 3). The effect size (i.e. mean change from base line to end of the treatment i.e. 6th cycle) of treatment in all parameters and stages showed a linear increase with increasing stages (last column, Table 3) i.e. treatment affected stage IIIA the least and stage IV the most. The oxidative damage in patients is evident more due to decrease (GSH: 27.5%; SOD: 22.8%) than increase (NO: 11.4%; LPO: 5.9%).

The two years overall survivals of all patients were summarized graphically in Figure 3. The overall mean (± SD) and median survival of patients were found to be 31.33±20.25 and 25.10 weeks respectively. Similarly, the stage wise survivals of patients were further sub-grouped and summarized graphically by Figure 4. Figure 4 showed that the median survival of patients of stage IIIA (median=65.10 weeks) were higher than the survival of patients of stage IIIB (median=24.35 weeks) and stage IV (median=22.20 weeks) while the survival between patients of stage IIIB and stage IV were almost same. On comparing, the survival proportions in patients of all three stage groups were found to be significantly ($\chi 2=7.17$,
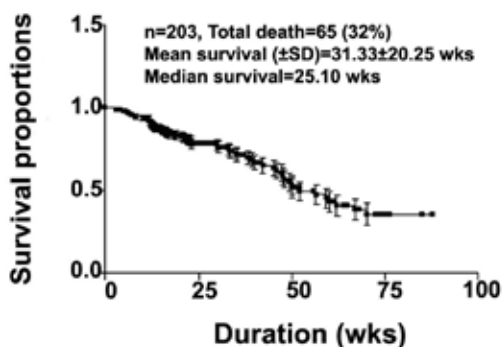

Figure 3. Graph showing Cumulative Survival (2 year) Proportions in Non Small Cell Lung Cancer Patients with SE (vertical bars)
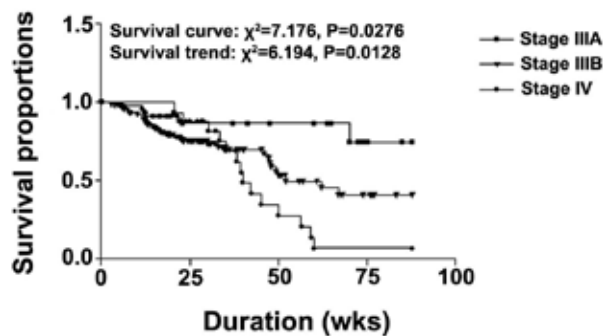

Figure 4. Graph showing stage wise survival (2 years) proportions in non small cell lung cancer patients with SE (vertical bars)

$P<0.05$) different. The trend analysis suggests that there were linear trends between stages and survivals ($\chi2=6.19$, $P<0.05$) i.e. the lower stage patients had higher survival than upper stage patients. However, the two year overall death proportion were maximum in stage IV patients (37.0%) followed by stage III B (31.7%) and III A (20.0%).

Discussion

Lung cancer is one of the most lethal cancers throughout the world owing to its high incidence and mortality. The incidence of Lung cancer is rising each year by 0.5%, resulting 1.04 million new patients each year worldwide (Maas et al., 2007). Depending on TNM stages at diagnosis, response rates to chemotherapy for inoperable NSCLC vary from 30-60% with platinum combined chemotherapy (Spira and Ettinger, 2004; Pfister et al., 2004).

Although platinum based chemotherapy have demonstrated clinical activity against NSCLC, advanced disease remains fatal with low response rates and short survival outcome (approximately 5% at 5 years, and <15% at 2 years) (Bunn and Kelly, 1998; Blackhall and Thatcher, 2004). The present findings are in agreement with these studies, which suggested that survival of stage III is high as compared to stage IV.

Tumor related factors (anatomic factors); Tumor size (T stage) anatomical structures involved (T4 vs. T3 lesions), the presence of regional lymph node metastasis have a significant impact on both prognosis and response to appropriate therapy. Goldie et.al 1979, also mentioned in their study that the chemotherapy response is related with illness stage, they showed in their model, response to chemotherapy is directly related with global tumour mass (Goldie and Coldman, 1979).

The high level of oxidative stress during chemotherapy may overcome the antioxidant defence of cancer cells resulting in lipid peroxide production and interfering with antineoplastic activity (Abdel-Aziz and El-Naggar, 1997). There is evidence that variety of anticancer drugs exert their cytotoxic activity by generating reactive oxygen species (ROS). These drugs include anthracyclines, such as cisplatin, etoposide adriamycin, bleomycin, cyclophosphamide (Look and Musch, 1994). These highly ROS can cause extensive tissue damage through reactions with all biological macromolecules. e.g. lipids, proteins, and nucleic acids leading to the formation of oxidized substances such as the membrane lipid peroxidation product malondialdehyde (MDA) (Halliwell and Chirico, 1993). Cisplatin based combination chemotherapy induces a fall in plasma antioxidant levels that may reflect a failure of the antioxidant defence mechanism against oxidative damage. This probably results from consumption of antioxidants caused by chemotherapy induced oxidative stress (Weijl et al., 1998).

Free radical induced lipid peroxidation is associated with membrane lipid destruction. (Esme et al., 2008) reported that increased lipid peroxidation was associated with clinical progression of the tumor. Our findings showed an increase in MDA levels in NSCLC as compared to control subjects, which are consistent with those of some previous studies (Seven et al., 1999; Bakan et al., 2002; Taysi et al., 2003), and also confirming the fact that as the stage of the lung cancer advances, levels of lipid peroxidation increases.

Nitric oxide (NO$^-$) has an unpaired e$^-$ and its derivatives produced by activated phagocytes may also play a role in the multi stage carcinogenic process (Ohshima and Bartsch, 1994). NO$^-$ is known together with other ROS to induce cytotoxicity and cytostasis. It has been found that the concentration of NO$^-$ under non-pathological conditions is at nanomolar levels and under condition of oxidant injury at micromole levels (Ma et al., 2000) and also shows cytotoxic and mutagenic effects (Taysi et al., 2003). NO$^-$ reacts rapidly with superoxide anion to form peroxynitrite, which may be cytotoxic by itself or easily decompose to the highly reactive and toxic hydroxyl radical and NO_2 (Nitrogen dioxide) (Ohshima and Bartsch, 1994). The present study revealed an increase in nitrite levels in patients with NSCLC compared with control subjects. In addition high level of nitrite was measured in advanced stages of lung cancer. It was also seen that the levels of Lipid peroxidation and nitrites were high in stage III after chemotherapy when compared to stage IV diseased NSCLC patients.

Glutathione is a well known antioxidant. The reduced form of the tripeptide glutathione is the most abundant low molecular weight thiol in all mammalian cell system (Gul et al., 2000). GSH protects cell against free radical injury and toxic effects of chemicals (Meister, 1984). SOD plays an essential role in the conversion of superoxide anion into H_2O_2 in the mitochondrial matrix, and a key survival factors in cells. For ex. SOD is a determinant of cell resistant to pro oxidant cytokines and contributes to the survival of cells against ionizing radiations and tumoricidal chemotherapeutic drugs (Kuninaka et al., 2000).

Although some studies have reported reduced antioxidant activities in the patients with lung tumor (Guner et al., 1996; Weijl et al., 1998), others have shown increased antioxidant activity in tumor cells (Zachara et al., 1997; Kaynar et al., 2005). The present study showed decrease in GSH and SOD in patients with advanced NSCLC as compared with control subjects. However it has been suggested that a decrease in antioxidant enzyme activity may be a response to increased ROS production, and in time, antioxidant enzyme activity may be inadequate for detoxification of high levels of ROS. In addition it was also observed that after chemotherapy the levels of SOD and GSH were low, when compared to stage III with stage IV NSCLC patients.

In conclusion, our study data suggests that as the lung cancer stage advances, the levels of oxidative stress increases. It was also observed after chemotherapy; oxidative stress increases with advanced stages, so that survival for patients in these late stages of disease remains poor. Although the major prognostic determinant for lung cancer is stage at presentation, there are differences in survival for patients with same stage disease. Therefore more accurate assessment of prognosis should be helpful in deciding therapeutic option for NSCLC patients.

References

Abdel-Aziz AF & El-Naggar MM (1997). Superoxide dismutase activities in serum and white blood cells of patients with some malignancies. *Cancer Lett*, **113**, 61-4.

Albain KS, Crowley JJ, Leblanc M & Livingston RB (1991). Survival determinants in extensive-stage non-small-cell lung cancer: the Southwest Oncology Group experience. *J Clin Oncol*, **9**, 1618-26.

Bakan E, Taysi S, Polat MF, et al (2002). Nitric oxide levels and lipid peroxidation in plasma of patients with gastric cancer. *Jpn J Clin Oncol*, **32**, 162-6.

Beutler E, West C & Blume KG (1976). The removal of leukocytes and platelets from whole blood. *J laboratory and clinical med*, **88**, 328-33.

Blackhall F & Thatcher N (2004). Chemotherapy for advanced lung cancer. *Eur J Cancer*, **40**, 2345-8.

Bunn PA, Jr & Kelly K (1998). New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. *Clin Cancer Res*, **4**, 1087-100.

Esme H, Cemek M, Sezer M, et al (2008). High levels of oxidative stress in patients with advanced lung cancer. *Respirology*, **13**, 112-6.

Evans JC, Ellman GL (1959). The ionization of cysteine. *Biochimica Biophysica Acta*, **33**, 574-6.

Goldie JH & Coldman AJ (1979). A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. *Cancer Treat Rep*, **63**, 1727-33.

Green LC, Wagner DA, Glogowski J, et al (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem*, **126**, 131-8.

Gul M, Kutay FZ, Temocin S & Hanninen O (2000). Cellular and clinical implications of glutathione. *Indian J Exp Biol*, **38**, 625-34.

Guner G, Islekel H, Oto O, et al (1996). Evaluation of some antioxidant enzymes in lung carcinoma tissue. *Cancer Lett*, **103**, 233-9.

Halliwell B & Chirico S (1993). Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr*, **57**, S715-24; discussion S724-5.

Jemal A, Siegel R, Ward E, et al (2007). Cancer statistics, 2007. *CA Cancer J Clin*, **57**, 43-66.

Kaynar H, Meral M, Turhan H, et al (2005). Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu-Zn superoxide dismutase activities, total glutathione, nitric oxide, and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer. *Cancer Lett*, **227**, 133-9.

Kumar S, Mohan A & Guleria R (2009). Prognostic implications of circulating anti-p53 antibodies in lung cancer--a review. *Eur J Cancer Care (Engl)*, **18**, 248-54.

Kuninaka S, Ichinose Y, Koja K & Toh Y (2000). Suppression of manganese superoxide dismutase augments sensitivity to radiation, hyperthermia and doxorubicin in colon cancer cell lines by inducing apoptosis. *Br J Cancer*, **83**, 928-34.

Look MP & Musch E (1994). Lipid peroxides in the polychemotherapy of cancer patients. *Chemotherapy*, **40**, 8-15.

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J biological chemistry*, **193**, 265-75.

Ma MK, Zamboni WC, Radomski KM, et al (2000). Pharmacokinetics of irinotecan and its metabolites SN-38 and APC in children with recurrent solid tumors after protracted low-dose irinotecan. *Clin Cancer Res*, **6**, 813-9.

Maas KW, El Sharouni SY, Smit EF & Schramel FM (2007). Sequencing chemotherapy, radiotherapy and surgery in combined modality treatment of stage III nonsmall cell lung cancer. *Curr Opin Pulm Med*, **13**, 297-304.

Mccord JM & Fridovich I (1969). The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J biological chemistry*, **244**, 6056-63.

Meister A (1984). New aspects of glutathione biochemistry and transport--selective alteration of glutathione metabolism. *Nutr Rev*, **42**, 397-410.

Mountain CF (1997). Revisions in the international system for staging lung cancer. *Chest*, **111**, 1710-7.

Mulshine JL & Sullivan DC (2005). Clinical practice. Lung cancer screening. *N Engl J Med*, **352**, 2714-20.

Novaes FT, Cataneo DC, Ruiz Junior RL, et al (2008). Lung cancer: histology, staging, treatment and survival. *J Bras Pneumol*, **34**, 595-600.

Nowak D, Janczak M (2006). Effect of chemotherapy on serum end-products of lipid peroxidation in patients with small cell lung cancer: association with treatment results. *Respir Med*, **100**, 157-66.

Ohkawa H, Ohishi N & Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, **95**, 351-8.

Ohshima H & Bartsch H (1994). Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res*, **305**, 253-64.

Parkin DM, Bray F, Ferlay J & Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Patterson RA & Leake DS (1998). Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less at acidic pH. *FEBS Lett*, **434**, 317-21.

Pfister DG, Johnson DH, Azzoli CG, et al (2004). American society of clinical oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol*, **22**, 330-53.

Relling MV & Dervieux T (2001). Pharmacogenetics and cancer therapy. *Nat Rev Cancer*, **1**, 99-108.

Sangeetha P, Das UN, Koratkar R & Suryaprabha P (1990). Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radic Biol Med*, **8**, 15-9.

Seven A, Civelek S, Inci E, et al (1999). Evaluation of oxidative stress parameters in blood of patients with laryngeal carcinoma. *Clin Biochem*, **32**, 369-73.

Spira A & Ettinger DS (2004). Multidisciplinary management of lung cancer. *N Engl J Med*, **350**, 379-92.

Taysi S, Uslu C, Akcay F & Sutbeyaz MY (2003). Malondialdehyde and nitric oxide levels in the plasma of patients with advanced laryngeal cancer. *Surg Today*, **33**, 651-4.

Weijl NI, Cleton FJ & Osanto S (1997). Free radicals and antioxidants in chemotherapy-induced toxicity. *Cancer Treat Rev*, **23**, 209-40.

Weijl NI, Hopman GD, Wipkink-Bakker A, et al (1998). Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. *Ann Oncol*, **9**, 1331-7.

Zachara BA, Marchaluk-Wisniewska E, Maciag A, et al (1997). Decreased selenium concentration and glutathione peroxidase activity in blood and increase of these parameters in malignant tissue of lung cancer patients. *Lung*, **175**, 321-32.