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Oxidant Stress and B Vitamins Status in Patients With Non-Small Cell Lung Cancer

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Abstract: *In this study, we examined oxidative stress and B vitamins status in non-small cell lung cancer (NSCLC) patients at different stages. NSCLC patients were divided into 2 groups, stage III (IIIA + IIIB, n = 27) and stage IV (n = 23). A total of 16 healthy control subjects were included for comparison. Plasma levels of α-tocopherol, β-carotene, vitamin C, Se, Cu, Zn, reduced glutathione (GSH), oxidized glutathione (GSSG), lipid oxidation and the activities of glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase, and xanthine oxidase (XO) were determined for evaluating oxidative status in these subjects. B vitamins (B_1, B_2, B_6, B_{12}, folate) in blood and plasma ghrelin level in these subjects were analyzed. Results showed that plasma level of ghrelin and lipid oxidation in NSCLC patients were significantly greater than control groups ($P < 0.05$). The activity of GPX, SOD, or catalase was significantly reduced, but XO activity was significantly elevated in NSCLC patients ($P < 0.05$). Plasma level of GSH was significantly lower, but GSSG level was significantly increased in NSCLC patients ($P < 0.05$). Vitamins B_2 and B_6 levels in red blood cells (RBC) from NSCLC patients were significantly lower ($P < 0.05$), and both were negatively correlated with plasma ghrelin. The correlation coefficients were –0.788 and –0.752, respectively. These data suggest that plasma GSH level may be a proper biomarker for evaluating oxidation status for NSCLC patients. RBC levels of vitamins B_2 and B_6 were reduced in NSCLC patients; thus, the importance of vitamins B_2 and B_6 for NSCLC patients could not be ignored.*

Introduction

Lung cancer is 1 of the major causes for cancer death in Taiwan and other countries (1–3). In Taiwan, about 5,500 to 7,800 new cases were diagnosed in 2001–05 (1). Human lung cancers are classified into 2 major types: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC); the latter is the most common type and accounts for around 80% of lung cancer cases (4).

It has been proposed that oxidative stress is involved in the etiology and deterioration of cancers (5–7), including lung cancer. The study of Kaynar et al. (8) reported that the activities of antioxidant associated enzymes such as catalase and glutathione peroxidase (GPX) in erythrocytes from NSCLC patients was changed, which affected the oxidation–antioxidation balance. Several nonenzymatic antioxidants such as α-tocopherol and ascorbic acid possess antioxidant property and play important roles in the antioxidant protection. However, the information regarding the contribution of these nonenzymatic antioxidants on antioxidative defense system in these NSCLC patients is lacked.

It has been documented that advanced lung cancer patients have suffered from undernutrition, weight loss, and even severe catabolic status such as cachexia (9–11). It may result from an imbalance between the activity of anabolic and catabolic hormones such as ghrelin and leptin. Ghrelin, a growth hormone-releasing peptide, is involved in metabolic regulation and favors a positive energy balance (12,13). The increased plasma ghrelin level in NSCLC patients with cachexia has been observed (9). However, it is unclear that ghrelin level in NSCLC patients changes without marked cachexia. On the other hand, B vitamins including vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6, vitamin B_{12},and folic acid are involved in many important physiological functions such as energy metabolism, cell reproduction, and cell membrane permeability. However, besides folic acid, less attention was paid to the variation of B vitamins in lung cancer patients, especially NSCLC patients.

The purpose of this study was to examine and compare the oxidant stress in NSCLC patients at different stages. In this study, we also examined the level of several nonenzymatic antioxidants and B vitamins in NSCLC patients without cachexia. The relationship between B vitamins and plasma ghrelin was also evaluated.

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Materials and Methods

Patients and Healthy Subjects

This study protocol was proved by Ethical Committee of the Medicine Faculty at Chung Shan Medical University. A total of 50 patients with cytologically or histologically confirmed NSCLC at Chung Shan Medical University Hospital between January and July 2006 were included in this study. These patients (26 male and 24 female with age range being 29–87 yr, mean age = 66.7) were taking no therapy and were newly diagnosed ones. These patients were nonsmokers and classified according to the international tumor-node-metastasis staging system: stages IIIA ($n = 12$), IIIB ($n = 15$), and IV ($n = 23$) for NSCLC. In this study, 50 NSCLC patients were divided into 2 groups as stage III (IIIA + IIIB, $n = 27$) and stage IV ($n = 23$). A total of 16 healthy control subjects confirmed no visible tumor by X ray (8 male and 8 female subjects with age range being 45–81 yr, mean age = 60.3) were also included for comparison. The data for body weight and height of patients and healthy subjects were collected.

Dietary Record and Nutrients Analysis

A 3-day dietary record including meal, snack, and drink was obtained from each subject. Nutrient composition was calculated by Nutritionist Professional software (E-Kitchen Business Corporation, Taiwan) and based on Taiwan Nutrient Databases (14).

Blood Sampling

Informed consent for study participation was obtained from 50 NSCLC patients and 16 healthy control subjects. A peripheral blood sample, 15 ml, from each subject was drawn after an overnight fasting. Plasma or serum was separated from erythrocyte immediately after blood collection.

Biochemical Measurements

Serum level of albumin, glucose, cholesterol, triglyceride, creatinine, and uric acid was determined by an autoanalyzer (Dr. Lange LP 420, Konisburg, Germany). Plasma immunoreactive ghrelin concentrations were measured in duplicate using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). The intraassay coefficient of variation (CV) was 8.1% and the interassay CV was 9%. Lactate dehydrogenase (LDH) concentration was determined in serum using photometric method by an automated instrument (Shimadzu CL-7300, Tokyo, Japan).

The plasma level of α-tocopherol and β-carotene was quantified by a reverse-phase high-performance liquid chromatography (HPLC) method (15). Vitamin C (ascorbic acid) level was determined by a fluorometric method (16). The plasma levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined by commercial colorimetric GSH and GSSG assay kits (OxisResearch, Portland, OR), respectively. The activity of catalase (CAT), Cu-Zn superoxide dismutase (SOD) and GPX in plasma was determined by catalase, SOD, and GPX assay kits (Calbiochem, EMD Biosciences, Inc., San Diego, CA). Xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm (17). Lipid oxidation level was determined by measuring the formation of malondialdehyde (MDA) via an HPLC method (18) in plasma. Plasma level of Se, Cu, and Zn was determined by flame atomic absorption spectrometry (Perkin-Elmer Model 5000; Perkin Elmer Cetus Instruments, Norwark, CT). The level of vitamins B_1, B_2, and B_6 in whole blood, plasma, or red blood cell (RBC) was determined by HPLC methods (19–21). Folate and vitamin B_{12} (cobalamin) were analyzed by radioproteinbinding assay (Bio-Rad Laboratories, Richmond, CA). For folate determination, folic acid as pteroylglutamic acid was used for calibration, and its ^{125}I-labeled analog was used as the tracer. For cobalamin determination, cyanocobalamin was used for calibration, and its ^{57}Co-labeled analog was the tracer for cobalamin assays.

Statistical Analyses

Data were subjected to analysis of variance, and differences with $P < 0.05$ were considered to be significant. Correlations between 2 variables were calculated by simple regression analysis (Minitab Inc., State College, PA).

Results

Dietary record was used for nutrient intake analysis. However, there was no significant difference in the nutrients concerned in this study among NSCLC patients and healthy control groups ($P > 0.05$; data not shown). The baseline characteristics in patients with NSCLC and healthy control groups are presented in Table 1. NSCLC patients at stage IV showed significantly lower albumin and cholesterol and higher uric acid than control healthy groups ($P < 0.05$). NSCLC patients also had significantly greater ghrelin and LDH levels than healthy control groups ($P < 0.05$) in which patients at stage IV had significantly greater ghrelin and LDH levels than patients at stage III ($P < 0.05$). Plasma lipid oxidation, determined as MDA level, was significantly increased in NSCLC patients in which patients at stage IV had significantly greater MDA level than patients at stage III ($P < 0.05$, Table 2). The plasma levels of α-tocopherol, β-carotene, ascorbic acid, Se, Cu, and Zn were similar between control healthy groups and NSCLC patients ($P > 0.05$). NSCLC patients showed significantly lower GSH and higher GSSG levels than healthy control groups in which patients at stage IV had significantly lower GSH and greater

Table 1. Baseline Characteristics in Healthy Control Group (Control) and Patients With Non-Small Cell Lung Cancer (NSCLC)[a]

		NSCLC	
Parameters	Control $n = 16$	Stage III $n = 27$	Stage IV $n = 23$
Body mass index (kg/m^2)	24.2 ± 2.3	22.1 ± 1.8	20.6 ± 2.5
Serum albumin (g/dl)	4.51 ± 0.46	4.06 ± 0.67	3.65 ± 0.73^b
Serum fasting glucose (mg/dl)	104 ± 17	113 ± 25	97 ± 20
Serum cholesterol (mg/dl)	221 ± 23	204 ± 26	176 ± 19^b
Serum triglyceride (mg/dl)	130 ± 21	106 ± 27	125 ± 18
Serum creatinine (mg/dl)	0.68 ± 0.17	0.75 ± 0.21	0.63 ± 0.24
Serum uric acid (μmol/l)	229.6 ± 15.7	271.8 ± 24.8^b	$357.4 \pm 30.1^{b,c}$
Serum LDH (U/l)	302 ± 20	403 ± 31^b	$467 \pm 36^{b,c}$
Plasma ghrelin (fmol/ml)	134 ± 16	179 ± 28^b	$234 \pm 32^{b,c}$
Other diseases			
COPD	0	0	0
Heart diseases	1	1	0
Liver diseases	0	0	1
Diabetes	1	2	1

a: Values are means ± SD. Abbreviations are as follows: LDH, lactate dehydrogenase; COPD, chronic obstructive pulmonary disease.
b: $P < 0.05$ versus healthy control group.
c: $P < 0.05$ versus patients at stage III.

GSSG levels than patients at stage III ($P < 0.05$). The activities of GPX, SOD, and CAT were significantly reduced in NSCLC patients in which patients at stage IV had significantly lower GPX and CAT activities than patients at stage III ($P < 0.05$). When compared with healthy control groups, XO activity in NSCLC patients was significantly elevated in which patients at stage IV had the highest XO activity ($P < 0.05$).

Blood levels of B vitamins are shown in Table 3. Significantly lower vitamin B_1 was presented in stage IV patients only ($P < 0.05$). When compared with healthy control groups, vitamin B_2, vitamin B_6, and folate levels in

Table 2. Plasma Level of Lipid Oxidation (MDA Level), Nonenzymic Antioxidants (Vitamin C, α-Tocopherol, β-Carotene, Glutathione), 3 Enzyme Associated Elements (Se, Cu, Zn), and the Activity of Antioxidant Associates Enzymes (GPX, SOD, CAT, XO) in Control and Patients With NSCLC[a]

		NSCLC	
Parameters	Control $n = 16$	Stage III $n = 27$	Stage IV $n = 23$
MDA (nmol/ml)	1.07 ± 0.34	3.56 ± 0.61^b	$5.43 \pm 0.88^{b,c}$
Vitamin C (μmol/l)	30.2 ± 3.3	28.6 ± 2.8	29.3 ± 3.5
α-tocopherol (μmol/l)	19.8 ± 2.5	18.9 ± 3.1	20.1 ± 2.7
β-carotene (μmol/l)	0.66 ± 0.11	0.71 ± 0.20	0.95 ± 0.23^b
GSH (μmol/l)	13.8 ± 1.4	7.96 ± 2.9^b	$5.42 \pm 2.3^{b,c}$
GSSG (μmol/l)	0.29 ± 0.13	1.76 ± 0.31^b	$3.32 \pm 0.95^{b,c}$
Se (μmol/l)	0.86 ± 0.13	1.14 ± 0.22	1.06 ± 0.19
Cu (μmol/l)	17.2 ± 2.6	15.7 ± 1.4	16.3 ± 1.9
Zn (μmol/l)	10.8 ± 0.6	9.4 ± 1.0	11.6 ± 0.8
GPX (U/l)	274 ± 18	147 ± 23^b	$88 \pm 13^{b,c}$
SOD (U/ml)	18.67 ± 0.19	12.29 ± 0.34^b	12.04 ± 0.68^b
CAT (U/ml)	10.77 ± 0.26	7.69 ± 0.31^b	$4.25 \pm 0.42^{b,c}$
XO (U/l)	1.2 ± 0.3	4.8 ± 0.7^b	$7.7 \pm 1.2^{b,c}$

a: Values are means ± SD. Abbreviations are as follows: MDA, malondialdehyde; GPX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; XO, xanthine oxidase; Control, healthy control group; NSCLC, non-small cell lung cancer.
b: $P < 0.05$ versus healthy control group.
c: $P < 0.05$ versus patients at Stage III.

Table 3. B Vitamins Level in Whole Blood, Plasma, or RBC from Control and Patients With NSCLC[a]

Parameters	NSCLC		
	Control $n = 16$	Stage III $n = 27$	Stage IV $n = 23$
Whole blood TDP (nmol/l)	104 ± 13	90 ± 18	71 ± 27
RBC TDP (ng/g Hb)	423 ± 26	409 ± 19	372 ± 21^{b}
Plasma FAD (nmol/l)	65.6 ± 2.4	55.3 ± 1.9^{b}	$42.7 \pm 3.4^{b,c}$
RBC FAD (nmol/g Hb)	2.73 ± 0.46	1.89 ± 0.23^{b}	$1.35 \pm 0.47^{b,c}$
Plasma PLP (nmol/l)	19.3 ± 1.0	16.6 ± 1.7^{b}	$11.0 \pm 2.2^{b,c}$
RBC PLP (pmol/g Hb)	309 ± 13	266 ± 21^{b}	$223 \pm 26^{b,c}$
Plasma vitamin B_{12} (pmol/l)	340 ± 28	335 ± 17	327 ± 3 1
Plasma folate (nmol/l)	30.5 ± 3.1	21.7 ± 2.7^{b}	19.4 ± 1.8^{b}

a: Values are means ± SD. Abbreviations are as follows: RBC, red blood cell; Control, healthy control group; NSCLC, non-small cell lung cancer; TDP, thiamine diphosphate; FAD, flavin adenine dinucleotide; PLP, pyridoxal 5′-phosphate.
b: $P < 0.05$ versus healthy control group.
c: $P < 0.05$ versus patients at stage III.

all NSCLC patients were significantly lower ($P < 0.05$) in which patients at stage IV had significantly lower vitamins $B_2$and B_6 levels than patients at stage III ($P < 0.05$). The relationships between plasma ghrelin level and RBC vitamin B_2 (or vitamin B_6) in 50 NSCLC patients are presented in Fig. 1. Both RBC vitamin B_2 and vitamin B_6 were negatively correlated with plasma ghrelin, and the correlation coefficients were –0.788 and –0.752, respectively. The correlation coefficient for the level of plasma folate and ghrelin was –0.564.

Discussion

Several studies have reported that NSCLC patients suffer from weight loss and even severe catabolic status such as cachexia (9–11), although all subjects who participated in our study did not develop marked cachexia. However, based on the gradually reducing body mass index and increasing LDH, the nutritional status of these NSCLC patients needs to be paid more attention. The study of Shimizu et al. (9) observed that lung cancer patients with cachexia had increased plasma ghrelin. The result of our study further found NSCLC patients without marked cachexia also had elevated ghrelin in plasma. Furthermore, we observed that more ghrelin was released in these NSCLC patients along with cancer progression from stage III to IV. Therefore, we thought the increased production of ghrelin was associated to catabolic status and occurred in NSCLC patients, but was not the result of cachexia. Because there is not sufficient data to explain why lung cancer increased ghrelin production, further study at the molecular level is necessary to elucidate the relationship between NSCLC and ghrelin.

Kaynar et al. (8) examined the oxidation status of NSCLC patients and found those patients had greater lipid oxidation level, and the activities of GPX, SOD, CAT, and XO in their erythrocytes were markedly elevated. In our study, we agreed that oxidation–antioxidation balance was altered in NSCLC patients, and the oxidative stress was elevated in NSCLC patients from stage III to stage IV, although our results observed the activities of GPX, SOD, and CAT in plasma from these NSCLC patients were reduced. We further noted that XO activity in plasma from NSCLC patients was elevated. It is known that XO is the rate-limiting enzyme in nucleic acid degradation; thus, the elevated XO activity apparently favored purine catabolism, probably in normal cells, which partially explained the increased production of uric acid in these NSCLC patients. The other explanation about the elevated plasma uric acid might be ascribed to the reduced excretion. On the other hand, XO could use molecular oxygen as an electron acceptor and generate superoxide anion and other reactive oxygen products (22). Thus, the elevated XO activity might consequently contribute to the enhanced oxidative stress in these NSCLC patients.

Many studies have indicated that nonenzymatic antioxidants such as vitamin C, α-tocopherol, and β-carotene could scavenge free radicals and suppress oxidation (23,24); thus, cancer patients are encouraged to intake these antioxidant nutrients (25–27). Because these NSCLC patients had severe oxidation stress, we expected to observe lower levels of these antioxidant vitamins in these patients. However, we found the blood levels of vitamin C, α-tocopherol, and β-carotene in these NSCLC patients were similar to those of healthy normal persons. Therefore, our results make us raise 2 questions: 1) Because these NSCLC patients still had sufficient vitamin C, α-tocopherol, and β-carotene, why could their oxidation stress not be improved? and 2) Do these NSCLC patients need to supply more antioxidant nutrients such as vitamin C or α-tocopherol? On the other hand, Chan et al. (27) reported that oxidized α-tocopherol (tocopheroxy radical) could be regenerated by ascorbate or GSH. Wang et al. (28) also indicated that the interaction of endogenous antioxidants such as ascorbic acid and GSH caused a

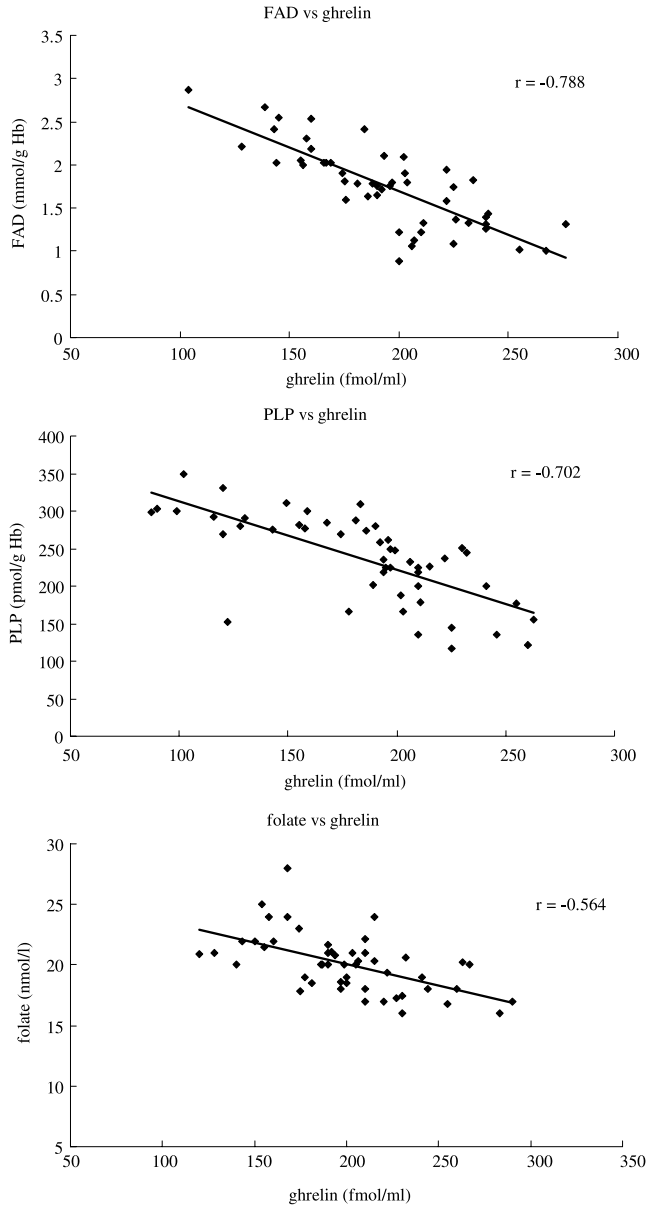

Figure 1. The relationship between plasma ghrelin (fmol/ml) and red blood cell flavin adenine dinucleotide (FAD; nmol/g Hb), pyridoxal 5′-phosphate (PLP; pmol/g Hb), and plasma folate (nmol/l).

compensatory network by which oxidative stress could be decreased. Thus, it is possible that α-tocopherol or vitamin C was continuously regenerated by GSH and acted as an oxidant scavenger in these NSCLC patients. This may partially explain the observed lower level of GSH and normal level of α-tocopherol or vitamin C. However, 1 question still remains: why these regenerated α-tocopherol or vitamin C failed to reduce oxidative stress because MDA level was still high. Since the decrease of GSH and increase of GSSG were along with cancer progression in these patients, these results suggest that plasma GSH level might be a proper biomarker for evaluating oxidative stress in NSCLC patients, and the intake of this agent might be helpful for alleviating oxidative damage.

The blood levels of vitamins B_2, B_6, and folate were reduced with lung cancer progression from stages III to IV. Vitamin B_2 is involved in nutrients' metabolism and influences epithelial integrity, prostaglandin biosynthesis, and GSH metabolism. Vitamin B_6 is a cofactor for many enzymes involved in amino acid metabolism and acts as a coenzyme responsible for the transfer of 1-carbon groups. Thus, the lower B_2 and B_6 levels in NSCLC patients might suggest the requirement for these B vitamins was increased for these patients due to the rapid energy metabolism and/or tumor growth. Further large-scale study is necessary to reverify the change in the parameters and to evaluate whether NSCLC patients need B vitamins supplements.

Recent studies have reported that vitamin B_6 has antiangiogenic and anticancer effects (29,30) because it could inhibit some types of eukaryotic DNA polymerases. The role of vitamin B_6 in the therapy of lung cancer, especially NSCLC, needs further studies to elucidate; however, the supplementation of vitamin B_6, at least, could help patients maintain normal physiological functions. It is interesting to find the relationship between plasma ghrelin and RBC vitamins B_2 and B_6. It seems more ghrelin was released when vitamins B_2 or B_6 levels was decreased in these NSCLC patients. It is postulated that more vitamins B_2 or B_6 will be obtained if these patients increase their food intake via the action of ghrelin. This inverse correlation between plasma ghrelin and RBC vitamins B_2 (or B_6) could be developed as an indicator for monitoring NSCLC progression.

In conclusion, this study provided several novel clinical findings regarding the status of oxidative stress and B vitamins in NSCLC patients. The blood GSH level may be a proper biomarker for evaluating oxidation stress in NSCLC patients. The levels of vitamins B_2 and B_6 in RBC were reduced in NSCLC patients, and these vitamins were inversely correlated with plasma ghrelin. Thus, the importance and influence of vitamins B_2 and B_6 for NSCLC patients could not be ignored.

Acknowledgments and Notes

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