Original Research Paper

Volume : 5 | Issue : 10 | October 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Assessment of Glutathione Status and Glutathione Peroxidase Activity in Lung Cancer Patients

Medical Science

KEYWORDS : Lung cancer, Glutathione, Glutathione Peroxidase, Reactive Oxygen Species

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ABSTRACT

Lung cancer is a leading cause of cancer-related mortality across the world, caring first place. It is the fifth common cancer in India. Cigarette smoke release excessive amount of free radicals such as Reactive Oxygen Species (ROS) which has the ability to induce oxidative stress and oxidative damage to the cells leading to cancer induction, a multi-step process. Oxidative stress elicited by aerobic metabolism, in human cells have developed a ubiquitous antioxidant defense system with Glutathione peroxidases (GPx), the primary cellular defense against ROS and reduced glutathione, to prevent cellular damage caused by peroxides and free radicals. Therefore the present study was focused to estimate plasma total reduced glutathione levels and glutathione peroxidase activity in lung cancer patients and controls. The study subject includes individuals newly diagnosed with lung cancer (n=50) and control (non-smokers) (n=50). Basic information such as age, sex, and smoking habit was sought using a questionnaire. The biochemical parameters were analyzed spectrophotometrically. The results of the study showed a significant reduction in the level of GSH among lung cancer patients and GPX activity was significantly increased in cases.

INTRODUCTION: Lung cancer is a leading cancer-related mortality across the world.

It is the fifth common cancer in India with

the number of new cases increased from around 65,000 in 2009 to 90,000 in 2013 and death due to lung cancer is projected to rise to ten million by 2030 (1). Lung cancer is the uncontrolled growth of abnormal cells that line the air passages. The association between lung cancer and smoking is well established, which increases the risk of lung cancer during their expected life time (2). Apart from carcinogens such as benzo[*a*]pyrene, cigarette smoke releases excessive amount of free radicals such as reactive oxygen species (ROS) which has the ability to cause massive injury to the cell. ROS cause oxidative stress and oxidative damage to the cell leading to cancer induction, which is a multi-step process (3).

Oxidative stress represents the inability of biological system to detoxify the ROS intermediates or to repair the resulting damage. Under normal conditions ROS is reduced into water and cells are protected against oxidative stress by an interacting network of antioxidant systems (4). The antioxidant defense system consists of endogenously-synthesized antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidant such as glutathione, vitamins, and micronutrients (5).

Reduced glutathione (GSH) is a linear tripeptide synthesized by the body (6). Glutathione the body's master antioxidant, found virtually in every cell of the human body. The highest concentration is in liver, making it critical in the body's detoxification process. It keeps other antioxidants such as vitamin E and ascorbic acid in their reduced states thus assist in neutralizing free radicals and ROS (7). Glutathione depletion has been correlated with lower immune function and increased vulnerability to infection due to the liver's reduced ability to detoxify (8). Glutathione Peroxidase 1 (EC: 1.11.1.9, GPx1), was the first identified mammalian selenoprotein (9). Glutathione Peroxidases (GPx) are critical intracellular enzymes involved in the reduction of hydrogen peroxide H_2O_2 to water and lipid peroxides to their corresponding alcohols using selenium as cofactor (10).

The objective of the present study was to relate the alteration in the antioxidant defense mechanism especially those of the glutathione system (Reduced glutathione and

Glutathione peroxidase) in lung cancer patients and controls (nonsmokers).

Materials and methods:

Study subjects: Blood samples of patients (n=50) newly diagnosed with lung cancer were collected from the Asirwatham speciality hospital, Madurai and from healthy volunteers (n=50) after getting their consent. The study was approved by the ethics committee of the hospital.

Reduced glutathione was estimated by the method of Moron *et al.*, (11) based on the development of intense yellow colour due to the formation of nitro-mercapto-benzoate anion from the reaction of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) with a compound containing sulfhydryl groups and the absorbance was read at 412nm for the plasma sample and a blank containing TCA. The amount of reduced glutathione was expressed as μM/mg of protein.

Glutathione Peroxidase activity was estimated by the method of Lawrence and Burk, (12). A known amount of enzyme preparation was allowed to react with (0.2 mM) H_2O_2 in the presence of reduced glutathione and 0.2 mM of NADPH for a specified period of 5 minutes. After initiation of reaction, oxidation of NADPH was followed at room temperature measuring the absorbance at 340nm using a spectrophotometer. A blank containing all reagents except plasma was also maintained. The activity of glutathione peroxidase was expressed as micromoles of NADPH consumed /min/mg protein. The protein concentration was determined by the method of Lowry *et al.*, (13) using bovine serum albumin as the standard.

Statistical analysis was carried out using, Sigma plot (v.11) software. The results of the biochemical analysis was subjected to Mann Whitney rank sum *U*- test to find statistical significance for the median values with inter quartile range and represented as Box plots.

Results:

The mean age of cancer patients was 52.86±1.38 years and that of controls was 41.0±1.45 years. The prevalence of lung cancer was 88% among men and 12% among women. The controls comprised of only men who were non-smokers, whereas 96% of the cancer patients were smokers (Table 1).

The median and interquartile range of reduced glutathione was significantly low in cancer patients when compared to that in controls (*P <0.001*). The activity of glutathione peroxidase was found to be significantly high in Lung cancer patients than in controls (*P=0.003*).

Discussion:

Lung cancer is the most prevalent and deadly malignancy worldwide (14). Siegel *et al*., 2014). The quantitative relationship between cigarette smoking and lung cancer mortality is formulated as a function of cumulative cigarette consumption. In addition to infection, certain medications and ageing, smoking can cause free-radical damage to healthy cells and deplete glutathione stores (15). In the present study a significant reduction in the level of reduced glutathione was observed among the lung cancer patients when compared to that of controls (*P<0.001*) (Fig. 1).

Parameters		Control (Non-smokers) (N=50)	Lung cancer patients (N=50)
Age (years) (Mean ±SEM)		41.0±1.45	52.86±1.38
Sex	Male	100%	88%
	Female	-	12%
Smoking Habit	Smokers	-	96%
	Non smokers	100%	4%
Reduced Glutathione * (µmole /mg protein)		133.33 (133.33– 190.0)	86.7 (86.66– 190.0)
Glutathione Peroxidase activity** (µM of NADPH consumed/min/mg protein)		4.09 (3.02-10.88)	7.415 (5.82-15.21)

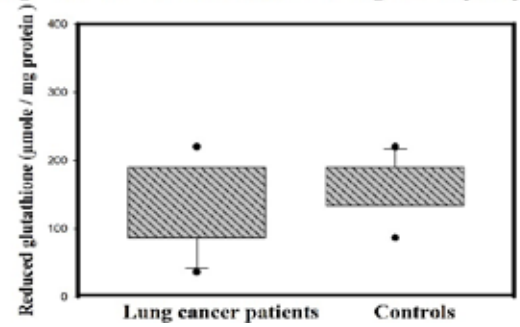
Table 1. Base line characteristics and Antioxidant status of the study subjects

Values represented as Median (Interquartile Range)

*- Statistically significant;***U** =751.5 (*P <0.001*);
** **U** =817.500 (*P =0.003)*

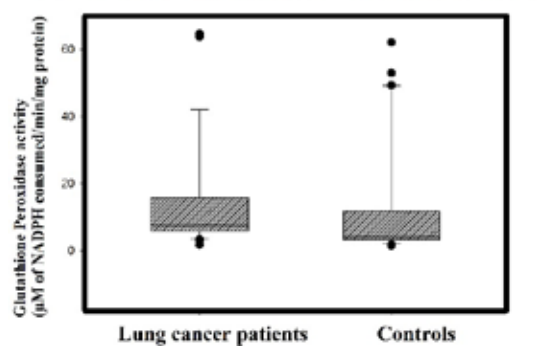
Also 96% of cancer patients in the study were smokers, and thus enormous amount of free radicals released by smokers contributed to oxidative stress leading to depletion of GSH. This indicates the free radical scavenging activity of the reduced glutathione, which tend to get depleted due to oxidative stress. Lowered GSH levels were reported in breast cancer patients which coincides with enhanced lipid peroxidation (16). Also a decrease in GSH level may be attributed to increased utilization to scavenge lipid peroxides as well as sequestration by tumor cells. In contrast to the present study reports are available for elevated levels of glutathione in tumor cells that are able to protect such cells in bone marrow, breast, colon, and lung cancer by developing resistance to chemotherapeutic drugs (17).

Fig. 1 Reduced Glutathione status among the study subjects



Several studies using primary cancer tissues reported an elevated levels of ROS-scavenging enzymes and antioxidant compounds (18). Glutathione peroxidase is a key enzyme in the defense against oxidative damage and its activity is correlated directly to cell survival (10). In the present study the glutathione peroxidase activity was found to be significantly high among lung cancer patients when compared to controls (*P=0.003*) (Fig.2). Elevated levels of GPx activity in cancer patient may be a marker of cell proliferation by eliminating H_2O_2 and other hydroperoxides. The enhanced GPx activity in the present study correlate with decreased GSH level in plasma of patients when compared to controls.

Fig.2 Glutathione Peroxidase activity among the study subjects



However, Robinson *et al*., (19) reported a decreased GPx activity with progression of neoplastic transformation in gastric cancer patients.

To conclude, the results of the present study revealed the antioxidant potential of reduced glutathione that counteracted the free radicals generated due to smoking, a risk factor for lung cancer. Furthermore, elevated levels of GPx was well correlated with the reduction in level of GSH, among lung cancer patients. Hence, further studies relating to the mechanisms regulating the expression and function of this crucial antioxidants to health and disease need to be unfolded.

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