

Research Article

Study of Nitrosative Stress and Role of Glutathione Redox System in Smokers

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Abstract: In the respiratory tract, NO[•] is generated enzymically by all three distinct isoforms of NO[•] synthase i.e. NOS-1, NOS-2 and NOS-3. Of these three forms NOS-2 activity is primarily regulated transcriptionally and is commonly induced by bacterial products and pro-inflammatory cytokines. Smokers are commonly characterized by an increased expression of NOS-2 within respiratory epithelial and inflammatory immune cells, and markedly elevated local production of NO[•]. The present study was planned to study glutathione peroxidase and reductase as well as nitrosative balance during inflammation in smokers. In this study 60 smokers with smoking history 20 pkts / year were included in the study. 100 healthy non-smokers were served as controls. Their base line clinical examination, nitric oxide (NO[•]), glutathione reductase (Rx), glutathione peroxidase (GPx) and vitamin C were measured. The results indicate that the mean nitric oxide levels in the patients at base line were high (P< 0.001) than Controls. The glutathione reductase (Rx), glutathione peroxidase (GPx) and vitamin C were low (P<0.001) in the smokers compared to controls. We found decreased levels of glutathione reductase (Rx), glutathione peroxidase (GPx) and vitamin C and increased levels of nitric oxide (NO[•]) in smokers. The nitric oxide level suggests that the total NO[•] production is high in smokers. The present study confirmed alteration in GSH/GSSG redox ratio.

Keywords: Nitric oxide, Glutathione reductase, Glutathione peroxide, Vitamin C, Smokers, RNS.

INTRODUCTION

Since its discovery as a biologic messenger molecule just over a decade ago, nitric oxide (NO[•]) has become well recognized for the participation in diverse biologic processes. High level of NO[•] can also cause respiratory tract injury and thus contribute to the pathophysiology of respiratory tract in smokers [1]. Lung cells are protected by extracellular GSH from oxidants produced and released by inflammatory cells and by intracellular GSH from oxidants generated in normal biochemical process as well as from xenobiotics [2]. Among the red cell antioxidants involved in detoxification glutathione play prominent role [3-5].

The human lung is one of the important storage areas of glutathione [6]. GSH is the essential cofactor for many enzymes which require thiol reducing equivalents and helps keep redox sensitive active site on enzymes in the necessary reduced state. Higher order thiol cell system the metallothioneine, thioredoxins and other redox regulatory proteins are ultimately regulated by GSH levels and the GSH / GSSG redox ratio. This balance is crucial to homeostasis stabilizing the cellular biomolecular spectrum and facilitating cellular performance and survival [7].

However, chronic inhalation of cigarette smoke was associated with a dramatic depletion of GSH [7,8]. Cigarette smoking may also affect critical detoxifying

and regulatory enzymes such as glutathione reductase, glutathione reductase involved in the GSH redox system. The lower respiratory tract is particularly sensitive to injury from inhaled and locally produced oxidants. GSH and its, redox system are important for the detoxification of toxic metabolites and lipid peroxides in smokers [1].

In healthy human subjects, NO[•] is known to originate from local synthesis by both constitutive and inducible forms of NO[•] synthase (NOS) present in several types (NOS-I, NOS-II, NOS-III) within the respiratory tract, including airway, alveolar epithelial cells, macrophages, neutrophils, mast cells, vascular endothelial and smooth muscle cells [9]. Although most exhaled NO[•] is now recognized to originate primarily from the nasal cavity and the sinus, NO[•] is known to be generated constitutively in all areas of the respiratory tract. Production of NO[•] is generally increased in smokers [10]. This enhanced production of NO[•] is thought to provide increased host defense against invading pathogenesis.

Excessive production of NO[•] during inflammatory diseases of the respiratory tract can also contribute to respiratory tract injury, which most likely involves the formation of more reactive nitrogen intermediates via interaction of NO[•] with inflammatory oxidants [11].

Despite the potential contribution of excessive NO• production to respiratory tract injury, protective effects of NO• against oxidant – induced cytotoxicity or lung injury have also been demonstrated in a number of investigation. These protective effects are most likely conferred by the ability of NO• to inhibit leukocyte activation and adhesion and to interface with radical mediated oxidative processes [12].

Study of the role of GSH redox system in protection against nitrosative stress and inflammation in the lung cells of smokers' not only will buffer antioxidant potential but may also inhibit inflammatory responses⁽¹³⁾

In the view of the above concept, this present study was planned to study glutathione redox system as well as nitric oxide balance during inflammation in smokers.

The following parameters were studied-

- Quantification of nitrite serum by assessing the lung damage induced alteration in nitric oxide concentration.
- To study possible alteration in glutathione peroxidase and glutathione reductase in smokers by Estimating the activities
- Quantification of the non enzymatic antioxidants to asses lung damage induced alteration in vitamin C

MATERIALS & METHODS

Serum Nitric oxide (NO.) as nitrite was estimated by Najwa Cortas and Nabil Wakid method [14].

RBC glutathione peroxidase activity was assayed by the method of Paglia and Valentine using Kits of Randox Co. U.K. [15].

RESULTS

Table 1: Illustrate the levels of NO•, GPx, GR & Vitamin C in the healthy controls and smokers

Sl. No.	Parameters	Healthy controls n=100	Smokers' n=60
1.	Sr.NO•(μmol/L)	33.15±6.13	137.58±16.19 *
2.	Glutathione peroxidase (U/L of Hb)	7213.77±58	2186.8±644.08 *
3.	Glutathione Reductase (U/L)	50.0±272	29.31±8.13 *
4.	Sr.Vitamin C (umol /L)	1253.12±170.22	411.09±72*

n = number of cases, All values are expressed in mean ± SD, * = Significant when compared with control group

In clinical examination the spirometry analysis of patients in the clinically stable phase of disease with FEV₁/FVC < 70 % were included. In the present study 60 smokers' both male or female and 100 controls between the age group of 25-75 years were included.

DISCUSSION

Table 1 displays serum nitric oxide (NO•) levels in healthy controls and smokers.

It was observed that the levels of serum nitric oxide measured as nitrite in the smokers were significantly elevated as compared to healthy controls (NO•) (P<0.001)

Plasma glutathione reductase activity was assayed by the method of Goldberg D.M. and Spooner R.J. using Kits of Randox Co. U.K. [16].

Plasma vitamin 'C' (Ascorbic acid) was estimated by the Caraway's Method [17].

Study design

Distribution of these subjects was as follows.

Sl. No.	Group	Types	No. of Subjects
1	Controls	Healthy subjects	100
2	Smokers'	Smoking history > 20 pkts /years	60

The control subjects were completely healthy non smokers and showed no abnormality on clinical examination including clinical history and spirometric analysis and were completely symptomless with no history or evidence of atropy or history of asthma . The study was cleared by institutional ethics

10 ml blood sample was collected from each patient 5ml of it was collected in EDTA bulb and 5ml was collected in plain bulb. Plasma and serum were separated from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

Statistical Analysis

The statistical analysis was performed by using student t test and P values < 0.001 were interpreted as statistically significant. The values were expressed as mean ± SD.

In smokers respiratory tract is commonly associated with elevated production of nitric oxide (NO•) and increased indices of NO• dependent oxidative stress are due to the formation of the oxidant peroxynitrite. The biochemical effect of NO• is largely defined by the concentration of NO•. The paramagnetic NO• molecule contains an odd number of electrons, which explains its highly reactive and radical nature. Autooxidation of NO• with O₂⁻ results in the formation of nitrite (NO₂⁻). However, at physiological concentrations of NO• and O₂⁻, this reaction may be too slow to be important in vivo. NO₂⁻ is also a substrate for hemeperoxidases such as MPO and EPO, which catalyze peroxidase

mediated oxidation and chlorination of biological targets. Moreover, peroxidase – catalyzed oxidation of NO_2^- results in the formation of a nitrogen dioxide radical (NO_2^\cdot) or related molecules.

The rapid reaction of NO^\cdot with free radical (radical-radical reaction) has emerged as one of the major routes to the formation RNS. At present the best understood of these reactions is the reaction with O_2^- to form ONOO^- , a strong oxidant. Although ONOO^- is relatively stable, it can be protonated to yield peroxynitrous acid (ONOOH), which then rapidly decomposes to NO_3^- via the intermediate formation of OH^- and NO_2^- . ONOOH is very unstable, highly reactive and capable of both oxidizing and nitrating reactions. For instance, invisible ONOOH modifications include nitration of aromatic amino acids, lipids or DNA bases. The amino acid tyrosine appears to be particularly susceptible to nitration and the formation of free or protein associated 3- nitrotyrosine has recently attracted interest as a potential biomarker for the generation of RNS in vivo.

Reactive Nitrogen Species (RNS) play important physiological functions and yet they can also cause extensive damage. The balance between physiological function and damage is determined by the relative rates of formation and the removal of ROS and RNS [18].

Excessive production of NO^\cdot in smokers in the respiratory tract can also contribute to respiratory tract injury, which most likely involves the formation of more reactive nitrogen intermediates via interaction of NO^\cdot with inflammatory oxidants [11].

The finding that nitric oxide level is high in smokers suggests that the total NO^\cdot production is high in humans with in smokers. Thus, smokers' are clearly subjected to nitrosative stress.

To prevent free radical formation or limit their damaging effects, Glutathione (GSH) – dependent antioxidant system protect against RNS and regenerate GSH from oxidized glutathione (GSSG) by the two important enzymes of glutathione redox system i.e. glutathione peroxidase and glutathione reductase.

Table No. 1 show glutathione peroxidase activity in healthy controls and smokers' significantly diminished GPx activity ($P < 0.001$) was observed in smokers'.

Glutathione (GSH) an important thiol, plays a major role in cellular protection against oxidative damage. Depletion of GSH renders the cell more susceptible to nitrosative stress. In addition to being a direct free radical scavenger, GSH is known to function as a substrate of glutathione peroxidase (GPx). Decrease in the activity of glutathione peroxidase in the present study may be due to increased reactive nitrogen species. Inhibition of superoxide dismutase leads to

accumulation of superoxide anion which in turn inactivates selenium-dependent glutathione peroxidase by its reaction with selenium at the active site of the enzyme.

Glutathione peroxidase is important selenium containing antioxidant enzyme which protect cells from peroxide damage. Selenium depletion decreases the activity of the GPx in smokers. Deficiency of glutathione peroxidase activity could result in increased tissue level of oxide such as nitric oxide. Increased levels of oxides could stimulate the activity of cyclooxygenase component of prostaglandin (PGH) endoperoxides synthase to increase thromboxane synthesis. Arachidonic acid is initially transformed into PGG₂. This transformation results from two sequential reactions, namely bisdioxygenation and hydroperoxidation.

During cyclooxygenation two molecules of O_2^- are incorporated into arachidonic acid to generate the intermediate PGG₂ that contains both a bicyclic endoperoxide and a hydroperoxide group. This peroxidase functions of more reactive oxygen radicals that could interact with polyunsaturated fatty acid in lung disease to form oxide [19].

Glutathione reductase is a homodimeric enzyme of which each subunit contains four well defined domains. The dimeric nature of the enzyme is critical for its functions because both subunits contribute essential residues to the constitution of the active site.

Smokers are exposed to high nitrogen attack and have diminished defense as indicated by elevated NO^\cdot levels and diminished GPx activity in these subjects.

Table No.1 depicts the levels glutathione reductase in healthy controls and smokers' significantly lower levels of glutathione reductase ($P < 0.001$) were observed in smokers' as compared to healthy controls.

It has been suggested that RNS may inactivate GR. The increased reactive nitrogen species brings about the conformational folding with burying of active site or molecular aggregation brought about by hydrogen bonding or disulfide bond formation. This decreases the activity of glutathione reductase enzyme in smokers [20].

Table No. 1 describe vitamin C in healthy controls and smokers. As compared to healthy controls vitamin C of smokers was significantly decreased ($p < 0.001$).

Ascorbate (vitamin C) is generally considered to be a key aqueous phase antioxidant and ascorbate deficiency may significantly contribute to nitrosative stress in smokers. This could be due to the fact that nitrosative stress causes more and more conversion of vitamin E to vitamin E radical and in order to re-generate vitamin E, vitamin C is used up leading to decrease in its level.

Plasma ascorbate may be a particularly important antioxidant in the plasma because the gas phases of cigarette smoke induces oxidation in plasma *in vitro* that is decreased by ascorbate [21]. Inhalation of NO[•] from cigarette smoke, as well as NO[•] and superoxide anion released by activated phagocytes react to form peroxynitrite, which is cytotoxic. Peroxynitrite has recently been shown to decrease plasma antioxidant capacity by rapid oxidation of ascorbic acid [22].

Ascorbate is generally considered to be a key aqueous phase antioxidant and ascorbate deficiency may significantly contribute to oxidative stress in lung disease patients. This could be due to the fact that oxidative stress causes more and more conversion of vitamin E to vitamin E radical and in order to regenerate vitamin E, vitamin C is used up leading to decrease in its level.

CONCLUSION

Our study confirmed the existence of nitrosative stress and alteration in glutathione reductase and glutathione peroxidase. It was found that decreased levels of non enzymatic antioxidants with increased levels of nitric oxide in smokers. It has been hypothesized that cigarette smokers were found to have altered glutathione redox system in their serum compared with non smokers. Therefore nitric oxide measurement may be used as a prognostic marker test for cigarette smoke dependent lung diseases.

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