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Studies on oxidative stress induced nerve conduction deficits in cigarette smokers

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Abstract

An important role of oxidative stress for the development of vascular and neurological complications has encouraged us to undertake a study to assess the oxidative stress induced nerve conduction deficits among cigarette smokers. Eighteen regular male cigarette smokers and twenty nine male non - smokers were diagnosed for clinical neuro-physiological tests *viz*., motor and sensory nerve conduction velocity (MNCV and SNCV) and redox status. Significant depletion of reduced glutathione (GSH) level ($p < 0.05$) and significant increase in malondialdehyde (MDA) level ($p < 0.01$) was found in smokers compared to non - smokers. Motor and sensory nerve conduction velocity showed no significant difference among smokers compared to non - smokers. The present study shows that smoking can induce oxidative stress among smokers but could not exacerbate to nerve conduction deficits.

Key words

Cigarette smokers, Oxidative stress, Nerve conduction deficit

Introduction

Cigarette smoking can cause a lot of impacts to the human body. Most important among them is the threat of the reactive oxygen species (ROS) (Northrop-Clewis and Thurnham, 2007). It was observed that increased oxidative stress can affect the nerve blood flow as well as nerve conduction deficits (Low and Nickander, 1991; Low *et al*., 1997). Nerve conduction studies are considered as the most sensitive indices of the severity of peripheral neuropathy, including alterations in function that are not recognized clinically (Tkac and Bril, 1998). Chronic cigarette smoking can induce alterations in membrane permeability properties of tissues and organs, which can result in changes in signal transduction and electrolyte imbalance (Padmavathi *et al*., 2009).

Recent study also reveals that smoking is associated with increased risk for ulnar neuropathy (Richardson and Jamieson, 2004). But no study is available on the role of oxidative insult on motor and sensory nerve conduction deficits among cigarette smokers. An important role of oxidative stress for the development of vascular and neurological complications by earlier study (Rosen *et al*., 2001) encourage us to undertake a study on the role of oxidative stress on nerve conduction deficits among cigarette smokers.

Materials and Methods

Eighteen male cigarette smokers and twenty nine male non-smokers with similar socio economic status were volunteered for the study from the city of Lucknow, Uttar Pradesh, India. The inclusion

criteria of the smokers: smokers with at least two cigarettes per day for the last 2 years. The exclusion criterion of smokers: (i) they should not smoke cigarette, beedi or other smokeless tobacco products and not using any alcoholic beverages, (ii) they should not have any medical complaints such as Diabetes mellitus. The inclusion criteria for non-smokers: (i) they should not smoke cigarettes, beedi or other tobacco products and not using any alcoholic beverages, (ii) they should not have any medical complaints such as Diabetes mellitus. The participants were selected from general population and those who hesitate/refuse to participate after detailed information regarding the study were excluded. Participants were not exposed to vibrating tools. Subjects with injuries to arm, carpal tunnel syndrome (based on Phalen's wrist flexion test) and nutritional deficiency based on medical examination, were excluded from the study. The selected participants were working as contract labourers doing cleaning and maintenance in the office and not occupationally exposed to metals, pesticides *etc*. Institutional Human Ethical Committee Clearance from Indian Institute of Toxicology Research, Lucknow, India was obtained.

Nerve conduction studies were performed on Electromyography Event Potential (EMG - EP) system (RMS, India). Recordings were obtained at following instrument settings: For motor studies: sensitivity: 2-5 mv mm^{-1}, low frequency filter: 2-5 Hz, high frequency filters: 10 KHz, sweep speed; 1-2 ms mm^{-1}. For sensory studies: sensitivity: 10-20 μv mm^{-1}, low frequency filter: 2-3 KHz, sweep speed: 1-2 ms mm^{-1}. Motor Nerve Conduction velocity (MNCV) as well as sensory nerve conduction velocity (SNCV) of median nerve of left forearm was measured using standard method (Ma and Levison, 1983). Electrodiagnostic reference value (Kimura, 1979) was considered as the normal values for the motor and sensory nerve conduction velocity and below normal values were considered as low MNCV and SNCV.

Biochemical parameters: Approximately 2 ml of venous blood was collected from both the smokers and non-smokers. The extent of lipid peroxidation in blood was assayed by measuring the formation of thiobarbituric acid reactive substances (TBARS) using standard method (Stocks and Dormandy, 1971) and values expressed as nmol MDA formed ml^{-1} blood using a molar extinction coefficient of 1.56×10^5 $M^{-1} cm^{-1}$. Reduced glutathione (GSH) was estimated in the whole blood by standard method (Jallow *et al.*, 1974) and oxidized glutathione (GSSG) in blood (Van Doorn *et al*., 1978) using Ellman's reagent and expressed as μmol ml^{-1} of blood. Catalase (CAT) activity was determined (Sinha, 1979) using H_2O_2 as substrate and expressed as μmol H_2O_2 hydrolysed min^{-1} g^{-1} Hb. For determination of superoxide dismutase (SOD) activity, we adopted the method (Misra and Fridovich, 1972) and expressed in m mol hydrolysed x 10^4 min^{-1} g^{-1} Hb.

Statistical methods: Significance of mean values of various biochemical and nerve conduction parameters in smokers and non - smokers were tested using student's *t* test after ascertaining the homogeneity of variances (Zar, 1984). Levels of significance were considered to be 5%.

Results and Discussion

Physical characteristics and smoking history of study subjects were given in Table 1. The antioxidant status of study subjects was shown in Table 2. GSH, CAT and SOD activities were found lower in smokers compared to non-smokers. MDA, GSSG level and GSSG/ GSH ratio were found higher in smokers in comparison to non-smokers. Significant depletion of reduced glutathione (GSH) level ($p<0.05$), and significant increase in malondialdehyde (MDA) level ($p<0.01$) was found in smokers compared to non-smokers. Nerve conduction studies among study subjects were represented in Table 3. There was no significant difference in MNCV and SNCV among smokers and non-smoker. Amplitude of SNCV as well as MNCV shows a lower value in smokers compared to non-smokers, but not found significant. Longer duration of latency for SNCV was observed in smokers corresponding to non-smokers.

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism (Pasupathi *et al.*, 2009). The human body has an inherent synergistic and multilevel defense mechanism, against the protection against ROS (Muzakova, 2001). Scavenger enzymes like SOD, catalase, glutathione peroxidase (GPx) and the non enzymatic antioxidant compounds like GSH and vitamins have the ability to inhibit oxidative stress by scavenging the highly destructive free radicals (Halliwell, 1994; Sreekumar *et al.*, 2001; Jyothi *et al.*, 2003). Cigarette smoke is a mixture of more than four thousand chemicals which is capable of undergoing complex interactions with human biological system (Cross *et al.*, 1999). The results of the present study show that there is a significant depletion of GSH and increase of MDA. But on other hand there is no alteration in the activity of SOD and Catalase. This may be due to the semiquinone components of tar in cigarette which reduce dioxygen forming superoxide radicals and hydrogen peroxide as reported in an earlier study (Witschi, 2005). Our study also

Table - 3: Nerve conduction studies among study subjects

Parameters	Motor nerve conduction velocity		Sensory nerve conduction velocity	
	Non-smokers (n=29)	Smokers(n= 18)	Non-smokers (n=29)	Smokers (n=18)
NCV (m sec^{-1})	51.52 ± 14.46	50.73 ±13.91	53.24 ± 11.06	52.53 ± 12.67
Amplitude (mV)	2.75 ± 2.04	2.71 ± 1.83	32.52 ± 19.77*	30.94 ± 29.15*
Prox. Latency (m sec^{-1})	2.47 ± 2.79	2.29 ± 2.60	2.89 ± 1.36	3.52 ± 2.12

Values are mean ± Standard deviation, * SNCV units expressed in μV

Table - 1: Physical characteristics and smoking history of study subjects

Parameters	Non-smokers (n=29)	Smokers (n=18)
Age (yrs)	29.34 ± 8.74	36.45 ± 14.40
Height (cm)	167.10 ± 7.31	170.25 ± 8.86
Weight (kg)	63.44 ± 9.75	64.03 ± 10.5
Cigarette smoking day^{-1}	—	3.39±1.72
Duration of smoking (yrs)	—	8.94±4.90

values are mean ± Standard Deviation

Table - 2: Antioxidant status among study subjects

Parameters	Non-smokers (n=29)	Smokers (n=18)
GSH (μ mol ml^{-1} blood)	21.61 ± 6.38	17.8 ± 4.52*
GSSG (μ mol ml^{-1} blood)	11.54 ± 3.21	12.31 ± 2.87
GSSG/GSH ratio	0.53	0.69
MDA (nmol TBARS ml^{-1} blood)	8.04 ± 3.52	11.72 ± 4.25**
CAT (μ mol H_2O_2 hydrolysed) X10^4 (ml^{-1} g^{-1} Hb)	72.88 ± 14.82	70.45 ± 19.11
SOD (m mol hydrolysed) X10^4 (ml^{-1} g^{-1} Hb)	1.44 ± 0.23	1.38 ± 0.25

values are mean ± Standard Deviation, significance at * $p<0.05$, ** $p<0.01$ against at non-smokers

supports earlier findings on the increase in the MDA and a decrease of GSH in cigarette smokers (Pasupathi *et al.*, 2009; Agarwal, 2005). Polyunsaturated lipids are particularly sensitive to free radical attack. The chain breaking peroxidation reaction generates conjugated dienes which further forms the hydroperoxides, commonly called thiobarbituric acid reacting substances or MDA (Jyothi *et al.*, 2003; Sreekumar *et al.*, 2001). GSH is the most sensitive antioxidant which protects the RBC from the insult, either through the glutathione cycle or the mercapturic acid pathway. So it can be assumed that toxic chemicals present in the cigarette increases the MDA thereby giving stress to the glutathione cycle. The failure of the GSH further brings about the remaining peroxidation insult in the body.

Reduced glutathione is a widely distributed cellular reductant and is a metabolic regulator and putative indicator of health. Blood glutathione levels are believed to be predicators of morbidity and mortality (Pastore, 2003). It plays a foremost role in protecting the cells from the electrophiles and free radicals. Reduced glutathione protects the body by scavenging the free radicals directly or by repairing damage to the structural or functional proteins by maintaining the SH group intact (George *et al.*, 2003). Smoking-induced depletion of GSH level is directly associated with elevation in lipid peroxidation which could be attributed to its protection against ROS generated by smoke, besides its consumption by the antioxidant enzymes GPx and glutathione S - transferase (GST). The acetaldehyde from the smoke conjugates with the GSH and thereby depleting the reduced glutathione pool in the cell, which further makes the cells more vulnerable to peroxidative damage (Waart *et al.*, 2000).

Our interest was to further see whether nerve conduction alterations occur due to smoking induced oxidative stress. But our study shows no significant effect on SNCV and MNCV among smokers. Earlier reports have demonstrated slowing of nerve conduction velocity in smokers (Husstedt *et al*., 1988; Kowluru *et al*., 1996). The role of oxidative stress on nerve metabolic defects was explained based on the disrupting nerve (Na, K) - ATPase activity (Kowluru *et al*., 1996), which lead to disruption of unmyelinated nerve fiber function. Sensory nerve action potentials and sensory conduction velocities are reduced in both axonal and demyelinating neuropathy (Olney *et al*., 1991). Smokers in the present study have not shown any significant change in nerve conduction, ruling out the possibility of axonal and demyelinating neuropathy in them irrespective of their oxidative insult.

Further, a large sample size study may substantiate the role of oxidative stress in nerve conduction alterations among cigarette smokers. To sum up, our present study suggests that cigarette smoke induce lipid peroxidation and the increase in peroxidation results in failure of the glutathione linked oxidative mechanism. However, it can be concluded that the oxidative stress has not lead to nerve conduction deficits. Since oxidative stress can lead to several co-morbid conditions in smokers, quitting cigarette will benefit smokers from health risks.

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