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RESEARCH ARTICLE

Laxmi Madhikarmi, The *Experiment, Feb, 2013 Vol. .7(3), 404-412*

ROLE OF CIGARETTE SMOKING ON LIPID PEROXIDATION AND ANTIOXIDANT PARAMETERS IN IRON DEFICIENCY ANEMIC INDIVIDUALS

ABSTRACT

Background

Cigarette smoking is a public health problem throughout the globe. Smoking is known to affect hemoglobin levels. Stress caused by cigarette smoke is known to cause serious diseases like cardiovascular diseases, hypertension, impairs immune system, stroke, irritable bowel syndrome, ulcer, etc. Iron deficiency is one of the most widespread nutritional deficiencies in the world.

Objectives

The present study was designed to investigate the effect of stress induced by cigarette smoke on lipid peroxidation and antioxidant parameters in patients with iron deficiency anemia.

Methods

The case-control study included sixty tobacco smoking iron deficient anemic patients and sixty healthy volunteers. The stress parameter was assessed by the determination of malondialdehyde and lipid hydroperoxide. The enzymatic and non-enzymatic antioxidants were assessed for total antioxidant status. An independent t-test was carried out to study the statistical significance and the level of significance was $p<0.05$.

Results

We found statistically increased lipid peroxidation and decreased antioxidants status, thus increasing the oxidative stress in smoking individuals. The present study showed that tobacco smoking is associated with increased oxidative stress, deteriorating the anemic condition.

Conclusion

Smoking is associated with the generation of increased free radical species which reduces the scavenging power of the antioxidants.

KEYWORDS Cigarette smokers, iron deficiency anemia, antioxidant, lipid peroxidation, oxidative stress.

INTRODUCTION

Smoking is a global public health problem associated with excessive morbidity and mortality [(1)]. Smoking caused 4.84 million premature deaths (2.41 million in developing, 2.43 million died in developed nation). According to World Health Organization (WHO) survey, 3.83 million male death and 1.0 million female deaths associated with smoking. According to Indian Council of Medical Research estimates, the annual estimated mortality due to tobacco-related diseases varies between 630,000 and one million [(2,3)].

Worldwide tobacco-attributable deaths were 4.83 million in 2000, projected to reach at 6.4 million in 2015 and 8.3 million in 2030. Worldwide, of 300 million young people who are smokers, 150 million will die of smoking related causes later in life. In the low and middle income countries such deaths are projected to increase from 3.4 million to 6.8 million between 2002 and 2030 [(3)].

Smoking is Iron deficiency anemia (IDA) with smoking habit are at an increased risk of complicated diseases due to increased free radicals produced by cigarette smoke leading to substantial reduction in life expectancy. Cigarette smoke contains 10^{14} free radicals/ inhalation. Nicotine present smoke disrupts mitochondrial respiratory chain leading to an increased generation of superoxide anion and H_2O_2 leading to oxidative damage of molecules including lipids, DNA, RNA, antioxidant enzymes; in subsequent cell through disruption of cellular function and integrity. The risk of diseases associated with smoking is related to the duration of the habit and quantity of cigarettes but cessation leads to a significant reduction in morbidity and mortality [(2, 4)].

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MATERIALS AND METHODS

We carried out age matched case-control study in a convenience sample of 120 male subjects in age group 20–50 years; 60 anemic and 60 healthy controls with their informed consent. 20–50 years age group of case-control was selected to avoid any age-dependent changes in relation to oxidative stress and antioxidant capacity. BMI was calculated by dividing weight (in kilograms) by height (in square meters). Blood samples (6ml) was collected after a 12–hr fasting period by veinpuncture and placed in sterile vial with EDTA as an anticoagulant.

2.1 Hematological parameter

Hemoglobin (Hb) levels in whole blood with anticoagulant was measured using Drabkin's reagent by cyanomethemoglobin reaction procedure (anemia in adult male Hb level <13g/dl and severe anemia is defined as Hb <7.0g/dl). All the spectrophotometric tests were determined using an UV-visible spectrophotometer (Shimadzu). Determination of red blood cells osmotic fragility was carried out based on the method described by Dewey et al. (1982) [(5)] with minor modifications as reported by Chikezie (2007) [(6)].

2.2 Determination of Iron and total iron binding capacity

Serum iron and iron and total iron binding capacity was assayed by Coral Clinical Systems kit method.

2.3 Lipid peroxidation/ Oxidant parameters

The lipid peroxidation was measure by the formation of malondialdehyde which was assessed by the Thiobarbituric acid reactive substance (TBARS) in whole blood by the method of Satoh (1978) [(7)], erythrocyte lysates by the method of Ohkawa et al (1979) [(8)] and plasma by the method of Buege and Aust (1978) [(9)] was estimated. Similarly, lipid hydroperoxides (LPHO) was determined in erythrocyte lysates and plasma by the method of Jiang et al (1992) [(10)].

2.4 Enzymatic Antioxidant parameters

The superoxide dismutase (SOD) was estimated in red blood cell (rbc) lysates by the method of Kakkar et al (1984) [(11)]. Catalase in whole blood (wb) and rbc lysates were determined by the method of Sinha (1972) [(12)]. Similarly, glutathione peroxidase in rbc lysates was determined by the method of Rotruck (1973) [(13)].

2.5 Non-Enzymatic Antioxidant parameters

The estimations of Vitamin A in plasma were performed by the method of Bessey et al (1946) [(14)], Vitamin C in plasma by the method of Natelson (1971) [(15)], Vitamin E in plasma by the method of Baker and Frank (1980) [(16)],Total antioxidant activity in plasma (TAA) were estimated by the method of Benzie and Strain (1996) [(17)], reduced glutathione in whole blood were determined by the method of Beutler et al (1963) [(18)] and total protein in red blood cells and plasma by the method of Lowry et al (1951) [(19)].

2.6 Statistical analysis

Data were processed by use of standard statistical software SPSS 13.0 (USA). Descriptive statistics were mean ± standard deviation. Results were analyzed using Student's t-test; p value <0.05 were considered significant.

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3. RESULTS AND DISCUSSION

The iron deficiency anemic patients were screened by assaying serum iron and total iron binding capacity was assayed by Coral Clinical Systems kit method. The osmotic fragility test was performed both in healthy and anemic individuals with smoking habit. The iron deficiency anemic individuals showed increased hemolysis than that of the healthy individuals are presented in figure: 1.

Our study group included sixty anemic individuals and sixty healthy individuals within 20-50 years. Among them thirty were nonsmoker and rest thirty were cigarette smokers (<5sticks/day) both anemic and healthy individuals. The study group included only male individuals. The lipid peroxidation parameters included thiobarbituric acid reactive substance (TBARS) estimation and lipid hydroperoxides (LPHO). The enzymatic antioxidants included catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The non-enzymatic antioxidants included vitamin A, C and E, total antioxidant activity (TAA), reduced glutathione (GSH) and total protein.

On case-control study, we found statistically increased BMI in control than case but within the normal BMI range both in non-smoker and smoker (<5sticks/day) groups as shown in table 1. In non-smoking case, hemoglobin (Hb) levels were lower than that of non-smoking control group which was statistically significant. Likewise, Hb also decreased in smoking anemic group as compared with the smoking control and a significant relation was obtained. The lipid peroxidation parameters TBARS (whole blood, red blood cell and plasma) and LPHO (red blood cell and plasma) was statistically increased in the non-smoking and smoking anemic case as compared with their respective counterparts at $p<0.005$. All enzymatic antioxidants (CAT, SOD, and GPx) showed statistically significant relation in non-smoking anemic individuals as compared to their counterparts. On the other hand, only GPx in smoking case showed significance at $p<0.05$ as compared with their healthy counterparts. All non-enzymatic antioxidant parameter showed significance at $p<0.005$ when non-smoker case was compared with smoking controls. Anemic smokers on comparison with their counterparts showed very few significance on non-enzymatic antioxidant parameters. Only vitamin A, E and TAA showed statistical significance.

The biochemical parameters of anemic patients with respect to age group: 20-35 yrs and 35-50 yrs were studied in table: 2. The age groups of case and controls were divided into two group and under them lipid peroxidation, enzymatic and non-enzymatic parameters were studied.

The anemic patients in both the age groups showed lower BMI and Hb than the control group which was statistically significant. Both TBARS as well as LPHO was highly increased in both the age groups. The enzymatic antioxidants, both in 20-35 and 35-50 yrs showed statistical significance. In 20-35 yrs age group, all the non-enzymatic antioxidant parameters showed significance except total protein in red blood cells. In age group 35-50 yrs, all the non-enzymatic antioxidant parameters showed statistical significance except total protein in red blood cells and plasma.

Smoking is associated with lower Hb levels, low body weight and various diseases (cardiovascular diseases, hypertension, impairs immune system, stroke, irritable bowel syndrome, ulcer, etc). Our study group also revealed lower Hb level and lower BMI than the control group. The smoking IDA patients showed increased lipid peroxidation and decreased non-enzymatic antioxidants. In smokers, the enzymatic antioxidant CAT was found to be increased than the nonsmoking case as well as healthy nonsmokers indicating increased reactive oxygen species. Likewise, SOD and GPx was decreased in IDA smokers than the IDA nonsmokers as well as healthy nonsmokers [1, 4, 20-22].

Cigarette smoke is a complex mixture of toxic agents, free radicals, redox cycling agents, cytotoxic aldehydes and other carcinogens like polycyclic aromatic hydrocarbons, benzopyrenes and nitrosamines [1, 4, 23]. Each puff of cigarette smoke contains 10^{14} low molecular weight free radicals which can directly or indirectly initiate and propagate lipid peroxidation [1, 4]. There are approximately

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4000 chemicals in cigarettes, hundreds of which are toxic. The ingredients in cigarettes affect everything from the internal functioning of organs to the efficiency of the body's immune system which is destructive and widespread. Forms of tobacco chewing include pan (piper betel leaf filled with sliced areca nut, lime, catechu and other spices chewed with or without tobacco), pan masala or gutka (a chewable tobacco containing areca nut), and mishri (a powdered tobacco rubbed on the gums as toothpaste). The World Health Organization predicts that tobacco deaths in India may exceed 1.5million annually by 2020. Tobacco use among women is prevalent in all regions of India (commonly in north, east, northeast and Andhra Pradesh) and among all sections overall 2.4% of women smoke and 12% chew tobacco.

Children living in the household with one or more tobacco-smokers experienced disorders of iron metabolism; hemoglobin formation, RBC metabolism led to the development of anemia during the early period of life. Passive tobacco smoking exposes young children to several toxic substances of tobacco related alkaloids (N-nitrosonornicotine and 4(methylnitrosamine)-1-(3-pyridyl)-1-butanone) are strong carcinogens a causative agent of human lung cancer [(23)]. A strong relation between childhood anemia and children aged 0-35 months was obtained, and passive smoking from both parents was three times as likely to suffer from anemia as children that were not exposed. Passively inhaling tobacco smoke experienced disorders of iron, hemoglobin, and red cell metabolism, leading to the early development of anemia [(4, 23)].

Khan et al (2009) [(24)] suggested that men living in the urban slum areas are more likely to smoke both cigarettes and bidis than their counterparts living in the urban non-slum areas even after controlling for many variables (age, education, marital status, religion, birth place and types of work). A study based on rural areas in Bangladesh reported that tobacco consumption was two times more among the poor individuals than the rich and the poorest households were twice more likely to smoke tobacco as compared to the wealthiest households in Bangladesh. Unhealthy lifestyles in adverse socio-economic conditions, social norms, cultural beliefs, neighborhood characters, poor environment, availability of cigarettes, and worse provision of preventive services in the deprived areas may have significant impact on behavior of individuals.

Singh et al (2007) [(25)] showed three fold higher prevalence of tobacco intake, among men compared with women, and all forms of tobacco consumption, i.e, smoking, chewing and smoking and chewing were more common among men than among women. Tobacco consumption among decedents in relation to age groups revealed that tobacco use by men was significantly more common in the age groups 35-44 years and >55 years of age. The present study showed the higher level of oxidative stress in 20-35 yrs age group as compared to 35-50yrs. The level of antioxidant levels also found diminished. The results also suggested that cigarette smoking anemic patients has an opposite effect on the antioxidant status whereas it has a synergistic relationship with the lipid peroxidation parameters. Despite the campaigning of adverse effects of cigarettes, smoking is prevalent in the society.

Ascorbate is considered to be the first antioxidant (first line of defense in plasma) and the most effective antioxidant in human plasma, and vitamin C inhibits the oxidation of low density lipoprotein in vitro [(1)]. It is also an important physiological antioxidant that helps to regenerate reduced antioxidative tocopherol from the tocopheroxyl radical. The reduced capacity of catalase, superoxide dismutase and glutathione peroxidase to neutralize reactive oxygen species results in an increased generation of hydroxyl radical, which initiates the peroxidation of polyunsaturated fatty acids. Cigarette smoke contains peroxyl radical (induces lipid peroxidation) and acetaldehyde (depletes the cells of reduced glutathione making the cells more vulnerable to peroxidative damage). Reduced glutathione is the chief constituent of the thiol pool and a vital intracellular scavenger of free radicals which reflects depletion of non-enzymatic antioxidant reserves. It has been revealed that smokers have poorer diets than non-smokers resulting in the deficiency of nutrients leading to nutritional deficiency [(3, 4, 20, 21)].

Circulating erythrocytes are particularly susceptible to oxidative damage as they are exposed to a high partial pressure of oxygen, have membranes rich in PUFAs and contain large amounts of iron that can potentiate a free radical reaction. Bidis have been reported to

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produce higher levels of carbon monoxide, nicotinic acid and tar than cigarettes and produce approximately three times the amount of carbon monoxide and nicotine as well as approximately five times the amount of tar than cigarette smokers. It may be possible that these substances in bidis produce a greater amount of free radicals, which cause more deleterious effects on lipid peroxidation, resulting in higher erythrocyte MDA levels and lower concentrations of SOD and ascorbic acid activity due to the utilization of these antioxidants for the scavenging of free radical generation. Smoking results in a reduced supply of circulating antioxidants in the body might be due to the creation of an extra demand for antioxidants through oxidative stress [21-24, 25-28]. The diets of smokers usually contain lower amounts of antioxidant-rich foods resulting in a reduced antioxidant nutrient status of smokers. Our findings provide evidence of increased oxidants and diminished antioxidants in iron deficient individuals with smoking habit.

Our research article included detailed investigations of lipid peroxidation status and enzymatic and non-enzymatic antioxidant study in male iron deficient anemic patients which is a positive plus point. But it includes few limitations such as our study includes no female case, small sample size, duration of smoking, type of cigarette, other tobacco products apart from cigarettes such as bidi, hukka, tobacco chewing, etc. Further study on females is being carried out in the laboratory.

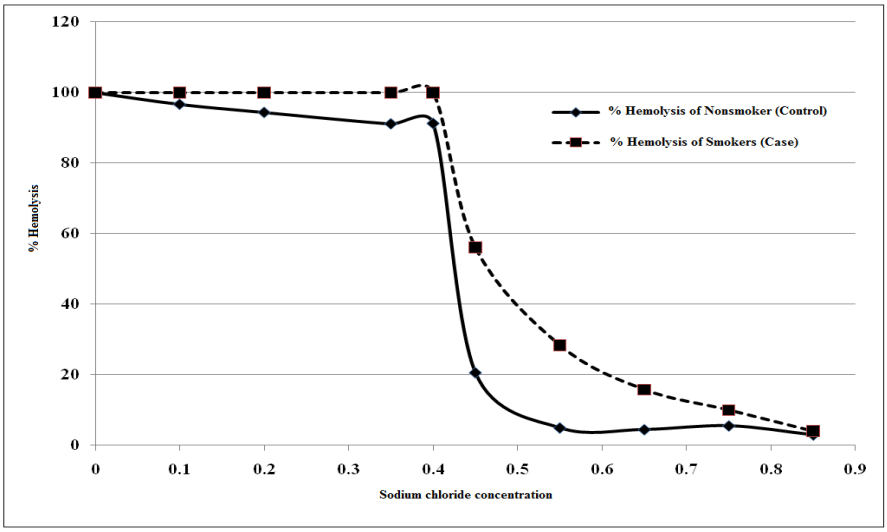

Figure 1. Osmotic fragility test of smoking anemic patients and controls.

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Table 1. Biochemical parameters of Iron deficient anemic patients with respect to Smoking index.

Parameters	Non-smoker		Smoker (<5sticks/day)	
	Case	Control	Case	Control
Age (years)	38.21±12.44**	32.24±.46	37.81±9.01**	35.42±8.88
BMI (Kg/m^2)	20.72±5.72**	22.18±2.47	20.16±5.12**	23.60±0.07
Hb (g/dl)	9.16±1.59**	15.27±1.59	8.97±1.19**	13.73±0.65
Iron (µg/dl)	38.26±1.66*	115.26±8.43	34.62±3.52*	95.79±12.41
TIBC (µmol/l)	98.46±2.83*	60.52±2.64	102.13±7.24*	65.62±8.65
Lipid peroxidation:				
TBARS-wb (nmol/ml)	5.25±0.20**	2.43±0.69	6.14±0.23**	2.67±0.17
TBARS-rbc (nmol/ml)	3.44±0.42**	1.81±0.33	3.84±0.20**	1.26±0.04
TBARS-p (nmol/ml)	5.11±0.14**	1.52±0.59	5.46±0.16**	1.67±0.12
LPHO-rbc (nmol/ml)	6.32±0.17**	3.02±0.19	6.42±0.61**	3.02±0.13
LPHO-p (nmol/ml)	3.55±0.17**	0.43±0.08	3.66±0.13**	0.43±0.20
Enzymatic antioxidants:				
CAT-wb (U/gHb)	1524.77±40.66**	512.69±23.18	1542.62±10.38	1116.89±20.85
CAT-rbc (U/gHb)	682.72±46.58**	416.69±28.12	685.42±65.37	687.99±29.90
SOD-rbc (U/mgHb)	37.30±5.70**	24.88±6.68	36.77±5.04	30.26±5.17
GPx-rbc (U/mgHb)	1.85±0.32**	3.46±0.06	1.35±0.71*	4.15±0.31
Non- Enzymatic antioxidants:				
VIT-p (µg/dl)	67.22±10.02**	83.63±12.42	56.13±6.93**	76.77±6.15
VITC-p (mg/dl)	0.42±0.18**	0.69±0.08	0.39±0.04	0.82±0.11
VIT E-p (mg/dl)	0.58±0.11**	1.35±0.48	0.47±0.11**	1.26±0.57
TAA-p (µmol/l)	546.78±13.77**	780.62±21.06	532.96±12.86*	772.00±12.24
GSH-wb (mg/dl)	22.87±3.30**	62.40±2.07	18.28±3.06**	49.33±5.18
TP-rbc (mg/dl)	38.16±9.12	37.40±8.91	36.99±9.99	34.60±5.90
TP-p (mg/dl)	66.85±3.77**	74.34±3.54	65.61±3.46	75.15±9.90

Note: **p<0.005, *p<0.05, wb-whole blood, rbc-red blood cells lysates, p-plasma

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Table 2. Biochemical parameters of Iron deficient anemic patients based on Age code.

Parameters	Age Code (20-35) yrs		Age Code (35-50) yrs	
	Case	Control	Case	Control
BMI (Kg/m^2)	22.27±6.77**	23.82±2.33	18.61±5.23*	21.96±3.4
Hb (g/dl)	9.34±1.22**	15.25±1.72	5.79±2.32**	13.75±0.89
Iron (µg/dl)	40.48±1.26*	118.23±8.21	32.40±3.82*	92.85±2.21
TIBC (µmol/l)	93.17±6.53*	52.76±2.81	107.42±2.44*	63.38±8.56
Lipid peroxidation:				
TBARS-wb (nmol/ml)	5.34±2.05**	2.81±0.08	6.05±3.23**	2.29±0.23
TBARS-rbc (nmol/ml)	3.43±1.63**	2.17±1.06	3.85±0.46**	0.91±0.86
TBARS-p (nmol/ml)	4.85±2.86**	1.52±0.57	5.72±1.59**	1.65±0.45
LPHO-rbc (nmol/ml)	6.08±2.85**	3.24±0.90	6.66±2.24**	2.82±0.43
LPHO-p (nmol/ml)	3.54±0.92**	0.42±0.16	3.67±0.67**	0.44±0.14
Enzymatic antioxidants:				
CAT-wb (U/gHb)	1448.66±47.10**	514.28±39.06	1618.73±26.47*	1115.30±40.58
CAT-rbc (U/gHb)	651.46±34.37*	529.38±26.53	715.68±23.46*	575.34±23.45
SOD (U/gHb)	47.48±14.90**	26.10±5.16	26.59±8.79**	29.04±2.64
GPx (U/gHb)	1.92±0.92**	2.71±1.42	1.30±0.11**	4.92±0.64
Non- Enzymatic antioxidants:				
VIT A-p (µg/dl)	58.18±10.66**	77.39±15.52	65.17±8.46**	83.01±3.48
VITC-p (mg/dl)	0.33±0.12**	0.68±0.16	0.48±0.16*	0.83±0.22
VIT E-p(mg/dl)	0.50±0.09**	1.63±0.42	0.57±0.034*	0.98±0.34
TAA-p (µmol/l)	543.52±17.33 **	829.20±16.62	537.22±3.45**	723.42±22.48
GSH-wb (mg/dl)	21.38±10.23**	51.02±1.5	20.77±9.67**	60.71±9.48
TP-rbc (mg/dl)	38.14±9.53	36.32±5.36	37.01±5.46	35.68±5.79
TP-p (mg/dl)	70.99±11.68**	82.21±9.50	61.47±11.49	67.28±7.89

Note: **p<0.005, *p<0.05, wb-whole blood, rbc-red blood cells lysates, p-plasma

4. CONCLUSION

Smoking has more effect on the health of men because it is associated with higher number of cigarette smoked. The present study indicates marked increase in reactive oxygen species production as reflected by elevated oxidant and decreased antioxidants parameters in IDA smokers. 20-50yrs time is the critical time for the health and future development and cigarette smoking can influence lifelong health risk. The main inducing factor for being addict is friends, hobby, influenced by parents, siblings, concentration, and personality symbol. Smoking causes greater decline in their capacities but these effects may be minimized by practicising physical activity and adopting healthier habits. Cigarette smoking should be banned globally because it is associated with paper waste (deforestation), land waste (agriculture), health hazard, addiction and environment pollution.

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