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ADULT HEALTHY SMOKERS;
LIPID PEROXIDANT AND ANTIOXIDANT ACTIVITY

1. MBBS, FCPS
Medical Officer
Department of Medicine
Liaquat University of Medical and Health Sciences Jamshoro
2. MBBS, M.D
Assistant Professor
Department of Medicine
Isra University Hospital Hyderabad.
3. MBBS, FCPS
Senior Registrar
Department of Medicine
Liaquat University of Medical and Health Sciences Jamshoro
4. MBBS, M. Phil, FCPS
Consultant Physician
Assistant Professor
Assistant Director Postgraduate Studies, Faculty of Medicine & Allied Medical Sciences, Isra University Hyderabad, Sindh, Pakistan.

Correspondance Address:
Dr. Haji Khan Khoharo
B. No: C-17/II, Phase I Anver Villas, New Wahdat Colony Qasimabad, Hyderabad, Sindh, Pakistan.
drhajikhan786@gmail.com

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Dr. Abdul Raqeeb[1], Dr. Azhar Memon[2], Dr. Mona Humaira[3], Dr. Haji Khan Khoharo[4]

ABSTRACT... Objectives: To determine superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA), uric acid and serum bilirubin in healthy adult smokers. **Study Design**: Case control study**. Place and Duration**: Liaquat University of Medical and Health Sciences Hospital. Hyderabad, Sindh from December 2013 - July 2014. **Subjects and Methods**: 77 smokers and 50 healthy controls were selected through non-probability purposive sampling. Blood glucose, lipids, MDA, antioxidant enzymes (SOD & GPX), serum bilirubin and uric acid (UA) were measured. Data was analyzed on Statistic software 8.1 by student's t test and Chi square test. The significant p-value was taken at ≤ 0.05. **Results**: Anti-oxidant enzymes (SOD & GPX), blood lipids, lipid per oxidant marker; the MDA, bilirubin and UA showed statistically significant differences between smokers and controls ($p<0.001$). Total blood lipids and lipid sub fractions were elevated in smokers. MDA in smokers was 3.17 ± 0.91 μmol/ml compared to 1.15 ± 0.61 μmol/ml ($p=0.001$) in controls. Smokers showed reduced SOD, GPX, serum bilirubin and UA, was significant ($p=0.0001$) in comparison to controls. **Conclusion**: Cigarette smoke is a significant source of oxidative stress. Smoking increases malondialdehyde and reduces superoxide dismutase, glutathione peroxidase, uric acid and bilirubin.

Key words: Smoking Superoxide dismutase Glutathione peroxidase Malondialdehyde

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INTRODUCTION

Smoking is a practice in which most commonly tobacco is burned in a paper wrap and the smoke is inhaled. This is primarily practiced as a route of administration, as combustion releases the active substances in cigarettes such as nicotine and makes them available for absorption through the lungs.[1]

Smoking is one of the leading causes of preventable death globally.[1] It has been suggested that smoking related disease kills one half of all long term smokers. A 2007 report states that about 4.9 million people worldwide die each year because of smoking.[2] Smoking has been shown a risk factor for atherosclerosis development and related complications such as cerebral and cardiovascular diseases (CVD).[2,3]

As cigarette smoke contains superoxide and reactive nitrogen species which readily react with various cell parts, hence, it is thought that most of the deleterious effects of smoking result from direct oxidative damage to endothelial cells and depleted nitric oxide synthesis.[4,5] Therefore, an imbalance between oxidants and antioxidants plays a role in smoking persons.[6] Cigarette smokers show enhanced inflammatory responses which exaggerate the oxidative stress.[7]

Since in humans, superoxide dismutase, glutathione peroxidase, uric acid and serum bilirubin are present in large quantities and exert anti-oxidant activity. They are an important intra- and extracellular free radical scavengers during metabolic stress including smoking.[8]

Measurement of malondialdehyde (MDA), anti-oxidant enzymes (SOD & GPX), uric acid (UA) and serum bilirubin is of clinical importance to assess the lipid per oxidant and anti-oxidant status in smokers. The present study hypothesized that smoking is independently associated with increased lipid peroxidation and reduced

antioxidant activity.

SUBJECTS AND METHODS

A prospective case control study was conducted at the Isra University, Hyderabad, Sindh from December 2013-July 2014. 77 smokers and 50 controls were selected through non-probability purposive sampling according to inclusion and exclusion criteria. Volunteer smokers of 20-50 years were recruited. Smokers with anti-platelet drugs, lipid lowering drugs, chronic disease of liver and kidney disease were excluded. A fasting of 8 – 12 hours was mandatory for taking blood samples. Obtained serum was pipette into a clean blood sample bottle and analyzed on the day of collection of blood glucose and lipid profile. Blood glucose, lipid and lipoprotein were measured by Roche Cobas e411 analyzer. Enzymatic colorimetric method; the CHOD-PAP and enzymatic – GPO-PAP method were used for detection of cholesterol and triglycerides respectively. precipitant method was employed for the detection of HDL-c and LDL-c by Friedewald's formula.[9] Thiobarbituric assay (TBARS) kit was used for the estimation of lipid per oxidant marker; the MDA. Glucose was detected by the glucose oxidase enzyme method.[10-12] Antioxidant enzymes (SOD & GPX) were measured by Elisa commercial assay kits. Bilirubin and UA were measured on the chemistry analyzer of Hitachi-Roche. Data was analyzed on *Statistic software 8.1* by student's t test and Chi square test respectively. The significant p-value was taken at ≤ 0.05.

RESULTS

The demographic characteristics are shown in Table-I. Controls were age and sex matched as measured by statistical analysis (p>0.05). Non-significant results were obtained for the blood glucose, uric acid, serum creatinine, obesity and BMI. Systemic blood pressure showed significant differences between cases and controls (Table-I).

MDA, blood lipids and antioxidants (SOD & GPX) showed statistically significant differences between cases and controls (p=0.001). MDA was raised 3.17±0.91 μmol/ml in smokers compared to 1.15±0.61 μmol/ml in controls (p=0.001). Lipids and lipid sub-fractions were elevated in cases compared to controls (p=0.0001) (Table-II). Table III shows the SOD, GPX, serum bilirubin and uric acid were reduced in smokers (p=0.0001).

	Cases (n=77)	Controls (n=50)	p-value
Age (years)	48±6.5	47±7.1	0.053
Male	58 (75.3%)	35 (70%)	0.19
Female	19 (24.6%)	15 (30%)	0.06
BMI (kg/m^2)	26±5.1	25±1.3	0.09
Obesity	38 (49.3%)	27 (54%)	0.08
Hypertension	53 (68.8%)	16 (32%)	0.001
Blood glucose (mg/dl)	153±41.0	113±51.1	0.06
BUN (mg/dl)	8±2.5	7±2.7	0.07
Serum creatinine(mg/dl)	1.2±0.43	0.9±0.33	0.03

Table-I. Characteristics of smokers and controls

	Cases (n=77)	Controls (n=50)	p-value
Triglycerides (mg/dl)	167.1±11.0	132.1±47.0	0.001
Cholesterol-Total (mg/dl)	158.1±39.7	118.3±21.9	0.0001
HDLc (mg/dl)	29.9±6.2	36.7±8.7	0.02
LDLc (mg/dl)	107.2±12.2	95.2±16.8	0.001
VLDL (mg/dl)	29 ± 9.2	28.9 ± 8.2	0.001

Table-II. Lipid profile of smokers and controls

	Cases (n=77)	Controls (n=50)	p-value
Superoxide dismutase (U/ml)	141.1±24.12	176.3±42.6	0.0001
Glutathione peroxidase (U/ml)	7519.5±134.0	8077.9±1019.0	0.0001
Bilirubin (mg/dl)	0.39±0.11	0.54±0.29	0.0001
Uric acid	3.14±0.78	4.01±1.9	0.0001
Malondial-dehyde (μmol/ml)	3.17±0.91	1.15±0.61	0.0001

Table-III. Anti-oxidants and Lipid per oxidant levels in smokers and controls

DISCUSSION

The present one is the first study being reported from a tertiary care hospital. The findings of malondialdehyde and anti-oxidant enzymes, the SOD and GPX, results were comparable to studies cited.[13-15] Natural anti-oxidant enzymes, the SOD & GPX, with a concomitant lipid per oxidant defect has been noted in smokers. Disturbed natural antioxidants and lipid per oxidant defects are strongly associated with cardiovascular diseases. Hence the finding of reactive oxygen species generated by smoking is supported by present study. The lipid peroxidation marker, the MDA was elevated in smokers in our study population. Above findings are in comparison to a previous study cited in literature.[13] It was reported that the ROS generation is increased with a concomitant reduction in enzymatic and non-enzymatic anti-oxidants increases the chances of vascular endothelial dysfunction which has been implicated in the cardio-vascular disorders,[14] and these derangements might be interacting in a synergistic way simultaneously to exaggerate one antoher.[13] A previously cited study[15] has reported that the non-enzymatic anti-oxidant factors such albumin, β-carotene, retinal, retinol, uric acid and α-tocopherol delay and inhibit the oxidative agents.[15] The present study also detected low levels of some non-enzymatic anti-oxidants, hence the present study concludes that the smokers are carrying high oxidative load. Our findings are comparable to cited studies as above.

Supporting body of evidence suggests a protective role of uric acid against cardiovascular disorders as it is claimed of exerting anti-oxidant activity.[16,17] Previous epidemiological studies had suggested that low serum uric acid is a risk factor for CVD.[18,19] In present study we found low serum uric acid which is comparable to above studies. The findings are in keeping with previous studies as regards low uric acid[4,20] and bilirubin.[21] Serum uric acid might be protective in conditions of increased cardiovascular risk and oxidative stress such as smoking, and by reducing its level it increases susceptibility to oxidative damage and generation of free radicals.[20-21] Hence, the possibility of uric acid confers protection against the atherogenesis has been recognized. Chronic smoking disturbs the natural endogenous antioxidants, i.e. the SOD, GPX, and uric acid thus increasing probability of cardiovascular diseases and recently the viability of administering uric acid has been established.[16] As shown in this study, smoking results in a reduced supply of circulating antioxidants in the body, which may be due to the creation of an extra demand for antioxidants through oxidative stress; this effect is directly correlated with amount of nicotine used per day by smokers.

Our current study is limited by some factors firstly, non-smokers controls might had history of smoking in remote past and we are not sure about history of degree of passive smoking. Secondly the effects of dietary factors which might affect the level of anti-oxidants were not assessed.

However, further investigations with large smoker sample size and analysis of dietary factor and other antioxidant factors are required to confirm the effect of smoking on the lipid peroxidation and antioxidants enzymatic and non-enzymatic systems.

CONCLUSION

Cigarette smoke is a significant source of oxidative stress in smokers. Smoking depletes the enzymatic and non-enzymatic natural anti-oxidants systems such as superoxide dismutase, glutathione peroxidase, uric acid and bilirubin, and increases the lipid peroxide load as estimated by malondialdehyde. Public awareness against cigarette smoking should urgently be launched by organizations working on the issue.

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"Your past was never a mistake if you learned from it."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Abdul Raqeeb	Concept, of study, Data analysis and manuscript writing and checking.	
2	Dr. Azhar Memon	Concept, of study, Data analysis and manuscript writing and checking.	
3	Dr. Mona Humaira	Concept, of study, Data analysis and manuscript writing and checking.	
4	Dr. Haji Khan Khoharo	Concept, of study, Data analysis and manuscript writing and checking.	