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CORRELATION OF MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE IN SMOKERS OF NORTH INDIAN POPULATION

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ABSTRACT : Smoking is the introduction of toxic substances into the body. Smokers inhaled hazardous compounds that are injurious to health. Currently, smoking is a global threat to public health, mainly in developing countries like India. The present study aimed to investigate the status and correlation of malondialdehyde (MDA) and superoxide dismutase (SOD) in smokers of North Indian population. In this case, a total of 60 subjects (30 smokers and 30 age-matched healthy non-smokers) were enrolled from in and around areas of Lucknow. The level of MDA and SOD activity were estimated in all subjects. For statistical analysis, student's t-test was performed between smokers and non-smokers. Pearson correlation coefficients were calculated among smokers. P value < 0.05 was considered statistically significant. The mean level of MDA was found significantly high in smokers as compared to non-smokers (P<0.001). The mean of SOD activity was found significantly low in smokers as compared to non-smokers (P<0.001). MDA and SOD have shown a significantly negative correlation among smokers (r= -0.47, P<0.001). Results showed that the smoking habit is the sole reasons for the oxidative stress in smokers. Raised level of MDA and declined SOD are the potential risk factors for chronic diseases. Cessation of smoking is required to reduce the burden of life-threaten chronic diseases.

Key words : Malondialdehyde, superoxide dismutase, smoker, non-smoker, chronic respiratory disease, North Indian population.

INTRODUCTION

Smoking is an act of burning tobacco substances and the generated smoke breathed inside the body. In the case of cigarette smoking, smoke contained a mixture of aerosol particles and gasses and alkaloid nicotine. Due to heating aerosol and gas are vaporized and these vaporized substances can be deeply inhaled and penetrated into the lungs where these active substances absorbed into the bloodstream. Cigarette smokers inhaled hazardous chemical compounds that are injurious to health. Presently, smoking is a threat to global public health, particularly in developing countries (HHS, 2014). Smoking is proven and recognized as lethally toxic to the human system. It is estimated that each cigarette tears away 7-11 minutes of human life (Jha *et al*, 2008). The study showed that tobacco smoking-related diseases are killing the general population approximately half of the long-term smokers than the average mortality rates faced by non-smokers. Smoking caused over five million deaths a year from 1990 to 2015 (Reitsma *et al*, 2017). It is projected to increase over eight million deaths yearly by 2030 (WHO, 2008) and more than 80% of the world's tobacco-related deaths will be seen in low- and middle-income countries (Mathers *et al*, 2006). Nearly, 0.8–0.9 million people die every year in India due to diseases associated with tobacco use (Reddy *et al*, 2004). According to National Health Family Survey (NFHS-3), India is projected to contribute 18% of tobacco-related deaths globally (NFHS-3, 2006; Gupta *et al*, 2008).

The Global Adult Tobacco Survey 2 (GATS 2) India 2016-2017 reported that overall 10.7 % (99.5 million) adults smoke tobacco (19.0% of men, 2.0% of women). Overall 28.6% (266.8 million) adults use tobacco (smoked and/or smokeless tobacco) (42.4% of men and 14.2% of women). In addition, adults were exposed to second-hand smoke at home, workplace and public place (38.7%, 30.2%

and 7.4%, respectively) (GATS, 2017).

The Global Adult Tobacco Survey (GATS) India 2009-2010 reported that more than one-third (35%) of adults in India use tobacco in some form or the other. There are about 120 million smokers in India only (GATS 2010). About 19% of tobacco consumption in India is in the form of cigarettes, while 53% is smoked as bidi (Gupta *et al*, 2008).

It is experimentally verified that cigarette smoke carries about 7,000 chemicals like toxic metals, poisonous gases and free radicals and of these 69 are carcinogenic (IARC, 2004; HHS, 2010). Amongst these, free radicals are regarded as most dangerous which carry unpaired electron which is highly reactive and cause oxidative damage to biomolecules and cell membranes (Phaniendra *et al*, 2015).

Smoking leads to the production of the massive amount of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl ion, superoxide, and peroxyl radical, which in revolve causes oxidative stress (Phaniendra *et al*, 2015). While cigarette smoke contains superoxide and reactive nitrogen species (RNS), which readily react with various cell parts, therefore, it is thought that the most of the harmful effects of smoking outcome from direct oxidative harm to endothelial cells and depleted nitric oxide synthesis (Griendling *et al*, 2016). Consequently, an imbalance between oxidants and antioxidants plays a key role in smokers (Birben *et al*, 2012). Cigarette smokers show enhanced inflammatory responses which exaggerate the oxidative stress (Li *et al*, 2013).

Generally, overproduction of ROS or a deficiency in endogenous antioxidant defense system, including enzymatic and non-enzymatic antioxidants, has been defined as oxidative stress (Birben *et al*, 2012). ROS initiates lipid peroxidation of cell membrane lipids resulting MDA. This aldehyde product is used as a biomarker to evaluate the level of oxidative stress in an organism ((Ayala *et al*, 2014). The increased levels of MDA due to lipid peroxidation is known to be crucial step in the pathogenesis of large number of pathological states like oral cancer (Shetty *et al*, 2014), lung cancer (Gegotek *et al*, 2016), asthma (EL-Alameey *et al*, 2017), COPD (Khan *et al*, 2019), Hypertension (Mishra *et al*, 2019), MetS (Bakhtiari *et al*, 2017), T2DM (Casoinic *et al*, 2016), CVD (Lee *et al*, 2012), atherosclerosis (Rafieian-Kopaei *et al*, 2014), cerebral aneurysm (CA) (Starke *et al*, 2018), CAD (Kaur *et al*, 2008) and pregnancy complications (Tiwari *et al*, 2016; Khan *et al*, 2019), summarized in Fig. 1. Therefore, MDA concentration in serum is a reliable biomarker to estimate lipid peroxidation status (Fogarasi *et al*, 2016). MDA sometimes may act as signalling messenger and regulating gene expression (Ayala *et al*, 2014).

SOD is the first enzyme in antioxidant resistance that scavenges superoxide radicals to form H_2O_2 and therefore diminishes the toxic effects of the radical. Decreased activity of SOD has been reported in pathological conditions. The quinine - semiquinone radicals from the tar phase of cigarette smoke are able of reducing molecular oxygen to superoxide radicals whose extreme production inactivates this enzyme. Hence, a decrease in SOD activity upon smoke exposure could have resulted

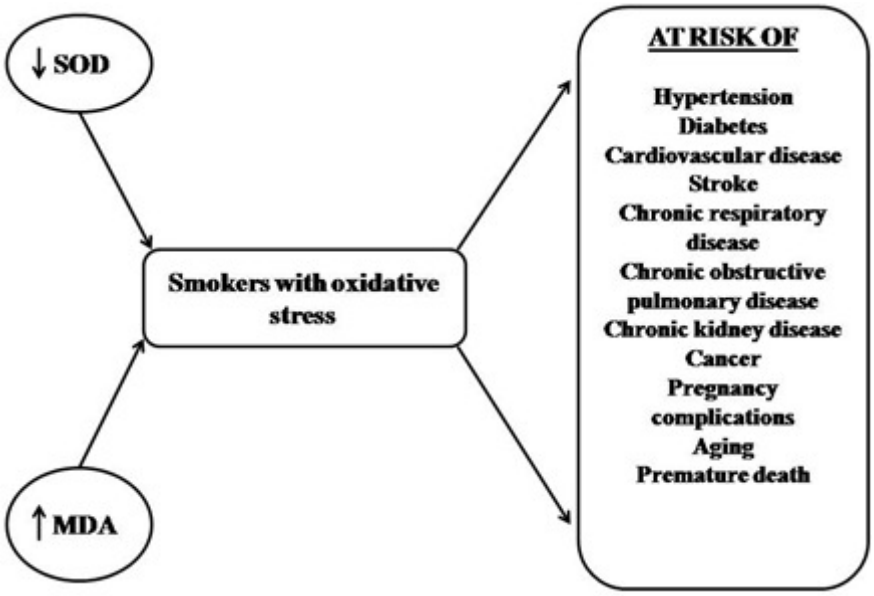

Fig. 1 : Diagrammatic representation of elevated oxidative stress and smoking associated risk.

from its inactivation by tar phase oxidants (Boukhenouna *et al*, 2018).

Hence, it is aimed to estimate the MDA levels and SOD activity to know the status of oxidative stress in smokers. This may be helpful to reduce the burden of chronic respiratory diseases in the general population.

MATERIAL AND METHODS

Subject selection

In this case-control study, a total of 60 subjects (30 smokers and 30 age-matched non-smokers) aged between 18-60 years was enrolled from outpatient Department of Medicine and Department of Pulmonary Medicine (TB & Chest) Clinic of IIMS&R, Integral University, Lucknow, Uttar Pradesh, India. The study was conducted out from January 2017 to June 2017. This study is approved by the institutional research and ethical committee and followed the ethical standards with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (WMA, 2013). Written informed consent was taken from each subject recruited for the study.

The inclusion of smokers and non-smokers: The person has a smoking history of bidi/cigarette and biomass fuels exposure from more than 02 years was considered a smoker. The person has not any smoking history of bidi/cigarette and biomass fuels exposure from less than/ more than 02 years were considered as a non-smoker.

Subjects with diabetes, ischemic heart disease, angina, Myocardial Infarction (MI), electrocardiogram abnormalities, anaemia (Hb of 8.0 g/dl or less), those with other concurrent sicknesses like the chronic liver disease, chronic kidney disease, hypothyroidism or those on drugs like antihypertensive, antioxidants and diuretics were excluded for both smoker and non-smoker groups. Detailed medical history was taken from each subject.

Laboratory investigations

About 3ml of venous blood was collected in EDTA vial from each subject for estimation of MDA levels and SOD activity. The plasma MDA levels were estimated by thiobarbituric acid reactive substance (TBARS) method (Ohkawa *et al*, 1979). Plasma was deproteinized and the precipitate is treated with thiobarbituric acid (TBA) at 90^0 C 1 hour. The pink color product formed at the end of the reaction which measured at 535 nm by UV-Visible double beam spectrophotometer (Systronics-2205, Systronic India Ltd. Gujarat, India). The SOD activity was estimated by the Nitroblue Tetrazolium (NBT) method (Durak *et al*, 1993). The assay of SOD is based on the inhibition of formation of NADH-phenazine methosulphate- nitroblue tetrazolium formazan. The color product formed at the end of the reaction, it was extracted into butanol and measured at 560 nm by UV-Visible double beam spectrophotometer (Systronics-2205, Systronic India Ltd. Gujarat, India). The enzyme activity (EA) unit is defined as the amount of enzyme that given 50% inhibition of NBT reduction in one minute; [01 Unit EA = 50% inhibition of NBT reduction in one minute].

Statistical analysis

Data analysis was performed using the IBM SPSS software version 20.0 (Armonk, NY, USA). All the data were compared between the two groups by using analysis of variance (ANOVA) or unpaired t-test. Values were represented as mean ± SD (Standard Deviation). Pearson correlation coefficient was calculated among smokers. A P value <0.05 was considered as statistically significant for all data analyzed.

RESULTS

The mean age of smoker and non-smoker were found 27.97±9.29 and 30.14±7.73 years, respectively (P=0.33). Similarly, the mean weight of smoker and non-smoker were found 55.70 ± 5.85 and 58.78 ± 8.58, respectively (P=0.12). The mean level of MDA was found significantly high in the smoker as compared to non-smoker (P<0.001). However, the mean activity of SOD was found significantly low in the smoker as compared to non-smoker (P<0.001) shown in Table 1.

A significant negative correlation was found between MDA levels and SOD activity among smokers (r = -0.47, P<0.01) shown in Table 2 and Fig. 2.

Table 1 : Anthropometric and clinical characteristics of smokers and non-smokers.

Parameters	Smokers (n=30)	Non-smokers (n=30)	P value
Age (years)	27.97 ± 9.29	30.14 ± 7.73	0.33
Weight (kg)	55.70 ± 5.85	58.78 ± 8.58	0.12
MDA (ìmol/l)	4.34±1.00	1.11±0.62	<0.0001*
SOD (U/mg protein/min)	1.23±0.30	2.80±0.86	<0.0001*

Values are expressed as Mean ± Standard Deviation *Significant considered as P<0.05.
MDA: Malondialdehyde, SOD: Superoxide dismutase.

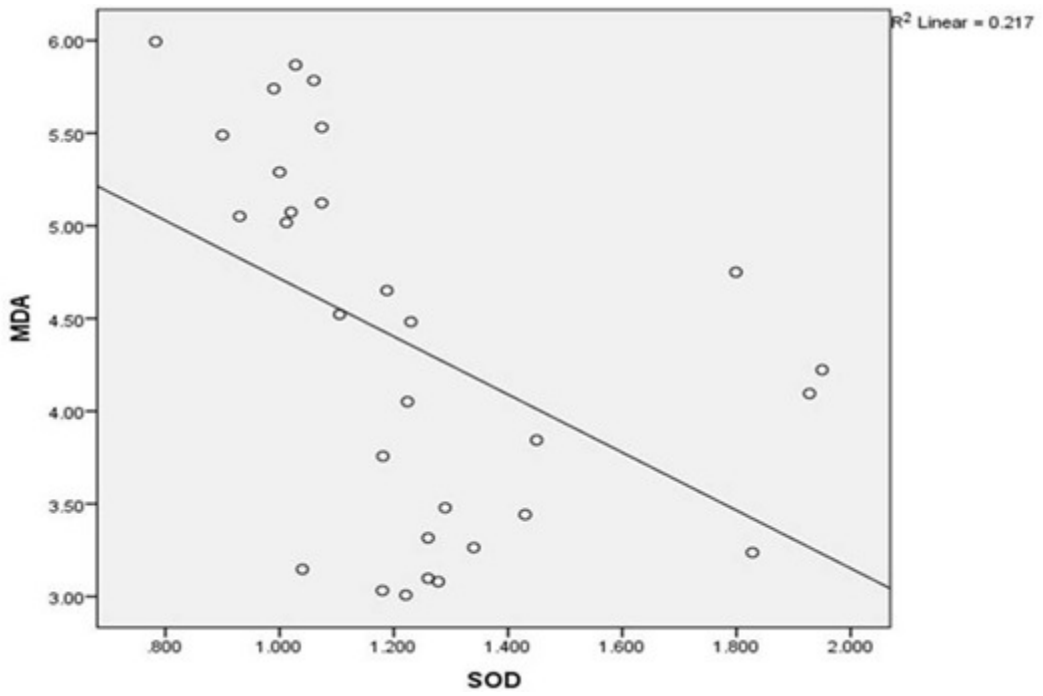

Fig. 2 : Correlation between SOD (U/mg protein/min) and MDA (ìmol/l) among smokers.

Table 2 : Correlation between variables in smokers

	MDA (ìmol/l)	SOD (U/mg protein/min)	Weight (kg)	Age (years)
MDA(ìmol/l)	1	-0.466**	0.300	0.031
SOD (U/mg protein/min)	-	1	-0.182	-0.286
Weight (kg)	-	-	1	-0.149
Age (years)	-	-	-	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).
MDA: Malondialdehyde, SOD: Superoxide dismutase

DISCUSSION

Results showed that the mean level of MDA was found significantly high in smokers as compared to non-smokers (P<0.001). Similarly, a study reported that lipid peroxidation increased in smokers (Kashinakunti *et al*, 2011). The free radicals produced by cigarette smoke attack in the cell membrane lipids which can result in loss of membrane integrity (Phaniendra *et al*, 2015). MDA is an end-product generated by decomposition of arachidonic acid and larger PUFAs through enzymatic or non-enzymatic processes (Ayala *et al*, 2014). Smoking plays an important role in disturbing the oxidant and antioxidant balance. Tobacco smoke contains abundant ROS and also activated neutrophils released due to smoking also add to the pool of ROS, which depletes antioxidant mechanisms leading to tissue damage (Domej *et al*, 2014). The MDA levels in bidi smokers were about 02 folds and 04 folds more in mild smokers and heavy smokers, respectively (Jain *et al*, 2007; Aziz *et al*, 2013).

The present study showed that SOD activity was significantly decreased in cigarette smokers as compared to healthy non-smokers (P<0.001). Similarly, it is reported that the SOD activity decreased in smokers as compared to non-smokers (Padmavathi *et al*, 2018; Findikli *et al*, 2018). The antioxidant enzyme SOD serves as a primary line of defense in destroying free radicals. SOD reduces the radical superoxide to form hydrogen peroxide and oxygen.

A significant negative correlation was found between MDA levels and SOD activity among smokers (P<0.01). Previous studies were reported that the levels of MDA in smokers are significantly higher as compared to non-smokers and the levels of anti-oxidant enzyme SOD is significantly lower in smokers as compared to non-smokers (Waseem *et al*, 2012; Padmavathi *et al*, 2018; Findikli *et al*, 2018).

Therefore, MDA and SOD levels can be used as markers of oxidative stress in smokers. Smoking generated oxidative stress should be kept in mind in the understanding of the pathogenesis of various smoking-

related diseases and their management. Because cigarette smoking contains various oxidants as oxygen free radicals and volatile aldehyde, these would be possibly the main responsible factors for the injuries caused to the biomolecules exposed to cigarette smoking (Ayala *et al*, 2014; Phaniendra *et al*, 2015).

It is reported that the risk of death in smokers depends on the number of cigarette smoked daily, the duration of smoking and the degree of inhalation. For daily heavy smokers (> 20 cigs/day), the risk of lung cancer associated death is 23 times higher in men and about 13 times higher in women than non-smokers. The risks for light smokers are lower than heavy smokers. For daily mild smokers (1-4 cig/day), the risk of developing lung cancer is 5 times higher in women (age 35-49 years) and 3 times higher in men than non-smokers (HHS, 2004; Bjartveit *et al*, 2005; HHS, 2008).

Cigarette smokers have an increased risk of CVD due to increased levels of oxidized macromolecules in both gas and tar phase. Blood of cigarette smokers normally display decreased antioxidant capacity and increased oxidized lipids as compared to non-smokers (Bloomer, 2007; Lee *et al*, 2012). Cigarette smoke generated oxidative stress plays an important role in the pathogenesis of atherosclerosis and cerebral aneurysm (CA) (Rafieian-Kopaei *et al*, 2014; Starke *et al*, 2018). Both smokeless tobacco users and smokers face a higher risk of dying from CVD than nonusers. It is estimated that smoking increases the risk of stroke 2 to 4 times, while regular smoking doubles the risk of stroke in men (Asplund *et al*, 2003; Saha *et al*, 2010). The prime causes of death from smoking are CVD (1.69 million deaths), COPD (0.97 million deaths) and lung cancer (0.85 million deaths) (Ezzati *et al*, 2003). Approximately 40 percent of tuberculosis deaths in the country are related to smoking (HHS, 2014; Jha *et al*, 2015). It is reported that smoking is related to increased aging, cell damage, and disease through the mechanism of oxidative stress (Liguori *et al*, 2018; Mamoshina *et al*, 2019). A previous study estimated that smoking increases the risk for CHD by 2 to 4 times, for stroke by 2 to 4 times, for lung cancer by 25 times in men and 25.7 times in women and for COPD 12 to 13 times (HHS, 2014), summarized in Fig. 1. Smokers have a potential risk for diseases that affect the heart and blood vessels. Even people who smoke fewer than five cigarettes a day can have early signs of CVD (HHS, 2014).

The study reported that Bidi smoking also having a very high risk for lung cancer even more than that of cigarette smoking. Bidi smoking is strongly linked with severe respiratory impairment, CVD and all-cause mortality (Prasad *et al*, 2010; Duong *et al*, 2017).

Smoking cessation (quitting smoking) is only a way to prevent smoking generated oxidative stress damage. Although, nicotine present in tobacco, bidi, and cigarettes makes the smoking cessation process often very prolonged and difficult. Nicotine replacement therapy can be helpful to quit smoking successfully. Strong motivation, will power and family support may also play a key role to quit smoking.

Tobacco Dependence Treatment Guideline (TDTG), started public awareness and benefits of cessation of smoking. This guideline suggested that from the moment quit smoking, within a half day: blood pressure and pulse rate drops to normal, the temperature of hands and feet increases to a normal, carbon-monoxide level in blood drops to normal and oxygen level in blood becomes normal. Within 1-2 days: chance of heart attack decreases and nerve endings start regenerating (ability to smell and taste begins to improve. Within 3 days: bronchial tubes relax, making breathing easier. Within 2 Weeks to 3 Months; circulation improves, lung function increases up to 30%. Within a half year: coughing, sinus congestion, fatigue and shortness of breath decrease, the lungs function better, as congestion reduces. Within 1 year: risk of CHD decreases to half that of a smoker. Within 10 years: the risk of dying from lung cancer is reduced to half. Within 15 Years: Risk of dying from a heart attack is equal to a person who never smoked (TDTG, 2011).

Similarly, HHS 2010 recommended that just after 1 year of quitting smoking; CVD risks cuts and chances of heart attack drops sharply. Within 2 to 5 years risk of stroke reduces. Within 5 years risks for mouth, throat, esophagus and bladder cancers drop by half. After 10 years risk for lung cancer drops by half (HHS, 2010).

Various studies have reported that smoking quitting by age of 40 years can reduce the risk of death 90% and by age of 30 years the health benefits equivalent to never smokers (Jha *et al*, 2015).

Limitations of the study

Our current study is limited by some factors firstly, non-smokers controls might have a history of smoking in remote past and we are not sure about the history of the degree of passive smoking. Secondly, the effects of dietary factors are required to confirm the effect of smoking on the lipid peroxidation and antioxidants were not assessed.

CONCLUSION

Results showed that the smoking habit is the sole reasons for the oxidative stress in smokers. Increased

level of MDA and declined SOD are the potential risk factors for chronic diseases in smokers. Cessation of smoking is required to reduce the burden of chronic diseases. Further studies are warranted to look insight and reveal the cause of life-threaten pathological conditions in smokers due to the smoking generated oxidative stress.

Discloure of potential conflicts of interest

Funding : This study was not funded by any funding agency or company.

Conflict of interest : Author M. F. Iqubal**,** S. Khan, M. Ahmad, R. Alam, M. M. Khan, V. K. Srivastava and G. Hussain declare that they have no conflict of interest.

Ethical approval : All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent : Written informed consent was obtained from all individual participants included in the study.

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