International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2013 Vol. 3 (2) May-August, pp.289-294/ Somwanshi et al.
Research Article

EFFECT OF CIGARETTE SMOKING ON LIPID PEROXIDATION IN SEMEN

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ABSTRACT
The present study was done to determine the effect of cigarette smoking on lipid peroxidation of sperm cell membrane & to analyze its effect based on MDA (malondialdehyde) levels. About 100 semen samples were studied for MDA level (malondialdehyde). Out of these 100 samples, 50 semen samples were from non-smokers (as controls) and 50 semen samples were obtained from cigarette smokers (as cases). The MDA levels between smokers and non-smokers were compared. Smokers showed significant increase in MDA level as compared to nonsmokers & statistically significant positive correlation was found between smoking index and MDA level. To conclude cigarette smoking leads to oxidative stress by free radical generation (Reactive oxygen species-ROS) by the mechanism of lipid peroxidation. There is linear relationship between lipid peroxidation and ROS production with cigarette smoking.

Key Words: *Cigarette Smoking, Oxidative Stress, Lipid Peroxidation, MDA*

INTRODUCTION
Male reproductive functions may be altered by chronic exposure to bioactive compounds of cigarette smoke, capable of crossing blood – testis barrier following systemic absorption (Paul *et al.*, 1989). Various cigarette smoke products such as cotinine, poly aromatic hydrocarbon, cadmium, benzopyrene, polonium – 210 and so many others, are proved to be mutagenic and carcinogenic (Potts *et al.*, 1999).
Cigarette smoking also leads to a condition called, as 'oxidative stress' by free radical generation.3 Oxidative stress is a condition with an increased rate of cellular damage induced by reactive oxygen species (ROS) The cellular generation of ROS was first observed in mammalian spermatozoa in late 1940's. The field then remained dormant for 30 years, until Thaddeus Mann and Roy Jones published a series of landmark papers in the late 1970's. They reported the importance of lipid peroxidation as a mechanism for damaging mammalian spermatozoa (Aitken *et al.*, 1995; Ravekar *et al.*, 2003). There is linear relationship between lipid peroxidation and ROS production (William and Ford, 2005). We have undertaken this study to find correlation between lipid peroxidation and cigarette smoking.

MATERIALS AND METHODS
The present study was conducted at S.R.T.R. Medical College and hospital, Ambajogai and Government medical college, Latur between the periods of June 2003 to January 2005. Semen samples were collected from pathology laboratories in above two institutes as well as from private fertility clinics and private pathology laboratories in Ambajogai and Latur. A detailed clinical history was taken from all cases (**50**) and controls **(50**).
All these 100 semen samples were studied for MDA level (malondialdehyde). The mean age of controls was 30.26 years (as controls) and mean age of cases was 32.22 years.
Selection of cases and controls was done by excluding:

1) Ex-smokers
2) Alcoholics
3) Tobacco chewers
4) Caffeine drinkers
5) Other pathological diseases like varicocele, diabetes, etc

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Design

Fifty cigarette smokers were divided according to smoking index, as below 100, between 100 –200 and above 200.

❖ Distribution of controls & cases according to smoking index

Table 1: Distribution of controls & cases according to smoking index

	Controls	Cases			
		<100	100-200	>200	Total
	50	28	16	6	50
TOTAL	***100***				

❖ **Smoking index (SI) (Sharma *et al.*, 1985)**

- SI is calculated by following formula -

  SI = number of cigarettes smoked per day × duration of exposure in years.

- Number of cigarettes smoked per day means average number of cigarettes smoked per day in last seven days.

Methodology

Estimation of semen MDA level (1, 1, 3, 3-tetra methoxy propane) (Bernheim *et al.*, 1948)

- Estimation is done by TBA – TCA (Thiobarbituric acid-trichloroacetic acid) assay.
- **Standardization:**

1) Stock standard MDA (164g / 1000ml) - Malondialdehyde is used as standard.

- For preparation of 10 μm solution of MDA.

a) 1.6 ml MDA is taken in 10 ml of distilled water (D.W.) this gives 1M of MDA……**A**

b) 0.1 ml from above mixture is put in 100 ml of DW, This gives 1 mM of MDA……**B**

c) 0.1 ml from **B** is put in 10 ml of DW. This gives 10 μM of MDA……**C**

Tricholoroacetic acid (TCA) 20% - Prepared as 20g / 100 ml of DW.

Thiobarbituric acid (TBA) 0.67% - Prepared as 0.67g / 100 ml of DW.

Procedure:

With above working standard, prepare various dilutions and make the additions as given in the following table of standardization scheme.

nmol/ml	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Standard(ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
DW ml	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
TCA 20% ml	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
TBA 0.67% ml	1	1	1	1	1	1	1	1	1	1

- The test tubes with above dilutions are heated in boiling water bath for 30 minutes followed by cooling in cold water for 10 minutes.
- Chromogen in extracted with 4.0 ml of n-butyl alcohol by vigorous shaking.
- Separation of organic phase is facilitated by centrifugation on 3000 rotations per minute (rpm) for 10 minutes.
- Absorbance is determined at 530 nm in calorimeter.
- A graph is plotted with MDA concentration in nmol/ml(X axis) and optical density reading obtained from calorimeter at 530 nm (Y axis) (Graph 1).

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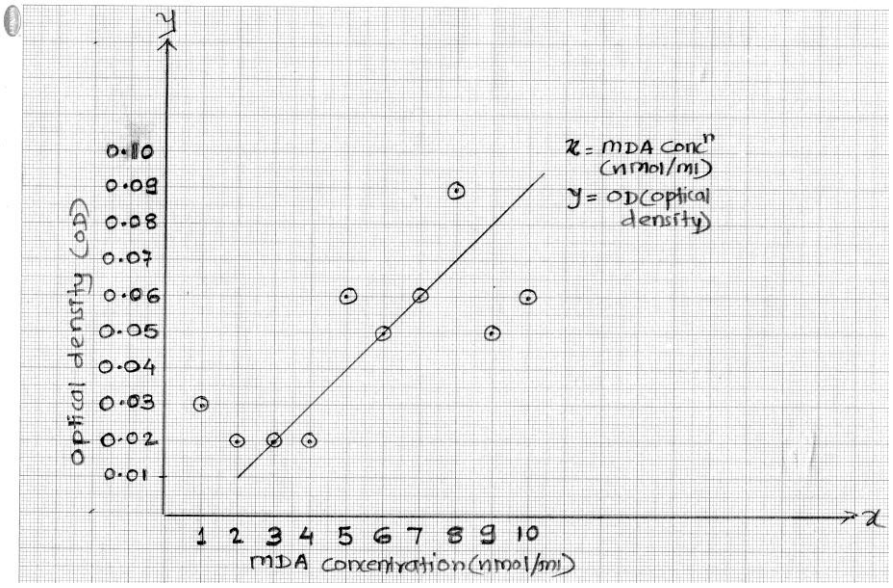


Method

- 0.5 ml semen is added with 2.5 ml of 20% TCA and 1 ml of 0.67% TBA.
- The mixture is heated in boiling water bath for 30 minutes followed by cooling for 10 minutes in cool water.
- Chromogen is extracted in 4ml n-butyl alcohol.
- Centrifugation is done at 3000 rpm for 10 minutes.
- Reading is taken at 530 nm in calorimeter.
- This optical density reading is matched with standardization graph and MDA concentration is obtained in nmols/ml

Statistical Analysis:

- Comparison of MDA level between smokers and non smokers is done by 'The test of significance of difference between two means'.
- Division of smoker group is done in three subgroups based on smoking index. To compare these three subgroups, for MDA level, 'Analysis of Variance' (ANOVA) test is applied.

RESULTS AND DISCUSSION

Results

Table 2: Comparison of MDA (nmol/ml) between non smokers (Controls) and smokers (Cases)

Groups	Control	Cases
No. of cases	50	50
Mean +S.D.	5.9 ± 1.77	6.64 ± 1.66
P value	$p<0.05$	
Significance	Significant	

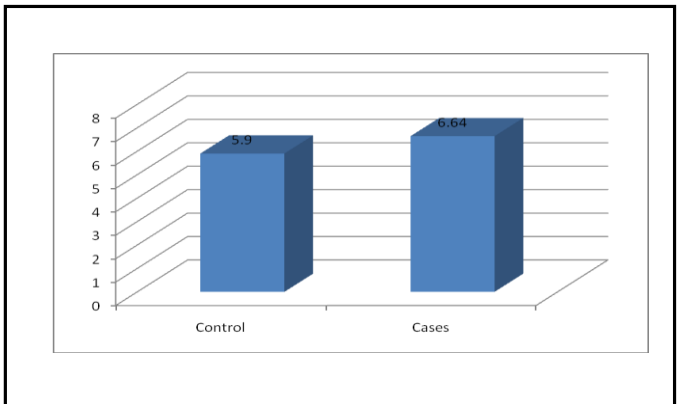
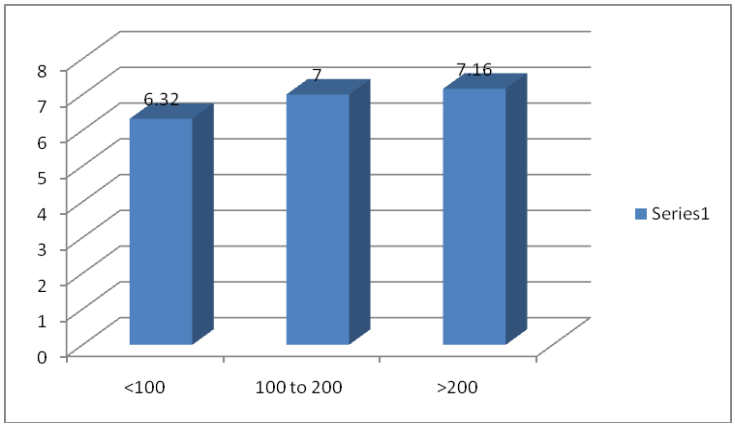


Table 3: Comparison of MDA level among smokers groups according to smoking index

Groups	<100	100-200	>200	Total
No. of cases	28	16	6	50
Mean + S.D.	6.32+1.25	7+1.35	7.16+0.66	6.64+1.66
F-ratio	F Value > 3.15			
Significance	Non Significant			



Our objectives to study the effect of cigarette smoking on lipid peroxidation of sperm cell membrane & to analyze the combined effect of number of cigarettes smoked per day and duration of exposure to cigarette smoking in years (Smoking index) on MDA level resulted as-

- Smokers (Mean MDA level = 6.64 ± 1.66) showed significant ($p<0.05$) increase in MDA level than non smokers (Mean MDA level=5.9 ± 1.77)
- Non significant (F Value > 3.15) positive correlation is found between smoking index and MDA level (Mean MDA level = 6.64 ± 1.66).

Discussion

Male reproductive function may be altered by chronic exposure to bioactive compounds of cigarette smoke components. These components, after systemic absorption, are capable of crossing blood – testis barrier and induce alteration in semen parameters and nucleus quality of spermatozoa (Poul *et al.*, 1989).

MDA Level

We found statistically significant increase in MDA level in semen of cigarette smokers as compared to non smokers (Table 2). Our study result matches with studies done by Koul *et al.*, (2001), Arabi (2004).

In contrast to our study, no association is found between smoking and MDA level as shown by Jozwik *et al.*, (1997).

Cigarette smokers show seminal leucocytosis (Sikka, 1996; Saleh *et al.,* 2003; Trummer *et al.,* 2002). Leucocytes are the source of ROS generation, which in turn causes DNA strand breaks (Potts *et al.,* 1999; Agarwal and Said, 2005). Cigarette smoke inhalation cause oxidative stress in tissues by free radical (ROS) generation (Koul *et al.,* 2001). Free radicals possess a dualistic role on sperm function. When produced in low levels, they enhance sperm function by stimulating DNA compaction and promoting a redox regulated C-AMP mediated pathway, central to induction to sperm capacitation. While when produced in excess, they result in DNA fragmentation, and peroxidative damage of sperm cell membrane (Sikka, 1996). Seminal plasma posses an antioxidant mechanism which is likely to quench ROS. But in certain conditions of oxidative stress like genitourinary infection, inflammation, ageing, cigarette smoking etc. This antioxidant mechanism may downplay which leads to excess free radical formation (Sikka, 1996).

Theoretically, cellular damage in semen is the result of an improper balance between ROS generation and scavenging activities eg: SOD, catalase, GSH-peroxidase and GSH- reductase etc (Sikka, 1996). These generated ROS cause lipid peroxidation of sperm cell membrane with generation of thiobarbituric acid reactive substances. End product is MDA (Potts *et al.,* 1999; Koul *et al.,* 2001; Rawekar *et al.,* 2003). Cadmium levels are found to be increased in cigarette smokers (Alexander *et al.,* 1998; Al-Bader *et al.,* 1999; Steven Sinclair, 2000). Cadmium crosses blood – testis barrier and can cause induction of free radicals (EL–Demerdash *et al.,* 2004). Thus it is clear that cigarette smoking results in lipid peroxidation of sperm cell membrane with increase in MDA level in semen.

Comparison of MDA Level among Smoker Group According to Smoking Index

Non significant positive correlation is found between smoking index and MDA level. This may be because of oxidative damage in smokers is due to the number of hours of active exposure to cigarette smoke (Altuntas *et al.,* 1989).

CONCLUSION

To conclude, the smokers showed statistically significant increase in MDA level than nonsmokers. Comparison among smoker group is done on the basis of smoking index.

Statistically non significant positive correlation was found between smoking index and MDA level. This is due to production of oxidative stress that results in to DNA fragmentation in sperm cell nucleus & peroxidative damage of sperm cell membrane leading to death of the cell with resultant reduction in sperm count as well as sperm motility.

Our study may prove to be helpful in investigating male infertility cases. If found positive, effect of quitting cigarette smoking on increasing sperm count – motility as well as it's rapidity of improvement & it's obvious relation to smoking index is matter of further research.

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