Original Article

# Effect of cigarette smoking on lipid peroxidation in semen, sperm count and sperm motility

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## Abstract

**Purpose**: Male reproductive functions may be altered by chronic exposure to bioactive compounds of cigarette smoke. Cigarette smoking also leads to a condition called as '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. We have undertaken this study to find correlation between lipid peroxidation and cigarette smoking. **Aims:** The present study is aimed to study the effect of cigarette smoking on lipid peroxidation of sperm cell membrane, sperm count and sperm motility and to analyze the combined effects of number of cigarettes smoked per day and duration of exposure to cigarette smoking in the years on MDA level and sperm count. **Methods**: About 100 semen samples were studied for sperm count and sperm motility. Out of these 100 samples, 50 semen samples were obtained from cigarette non smokers (as controls) and 50 semen samples are obtained from cigarette smokers (as cases). Comparison of MDA level between smokers and non smokers is done by 'The test of significance of difference between two means'. Comparison of sperm count and sperm motility between smokers and non smokers was respectively done by 'The test of significance of difference between two means' and 'Chi-square test'. Division of smoker group was done in three subgroups on the basis of smoking index. To compare these three subgroups, for sperm count and MDA level, 'Analysis of Variance' (ANOVA) test is applied. **Results:** Smokers showed statistically significant increase in MDA level than nonsmokers. A statistically significant decrease about sperm count and motility was found in smokers as compared to nonsmokers. While statistically significant positive correlation between smoking index and sperm count is found and statistically non significant positive correlation was found between smoking index and MDA level.
**Keywords:** Hypothyroidism, T4, T3, TSH.

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Received Date: 16/08/2016 Revised Date: 21/08/2016 Accepted Date: 11/09/2016

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.statperson.com |
| | DOI: 15 September 2016 |

## INTRODUCTION

Male reproductive functions may be altered by chronic exposure to bioactive compounds of cigarette smoke, capable of crossing blood – testies barrier following systemic absorption.[1] 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.[2] 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.[4,5] There is linear relationship between lipid peroxidation and ROS production.[6] We have undertaken this study to find correlation between lipid peroxidation, sperm count- sperm motility and cigarette smoking.

How to site this article: Sachin Devidasrao Somwanshi, Sunita Handargulle, Baby Minakshi Sable. Effect of cigarette smoking on lipid peroxidation in semen, sperm count and sperm motility. *MedPulse – International Medical Journal.* September 2016; 3(9): 838-841. http://www.medpulse.in (accessed 18 September 2016).

## METHODS

### Subjects

The present study was conducted at S.R.T.R. Medical College, Ambajogai and Government medical college, Latur between the periods of June 2003 to January 2005. Semen samples were obtained from pathology laboratories in above two institutes as well as from private fertility clinics and private pathology laboratories in Ambajogai and Latur. About 100 semen samples were studied for sperm count and sperm motility. Out of these 100 samples, 50 semen samples were obtained from cigarette non smokers of mean age 30.26 in years ( as controls) and 50 semen samples are obtained from cigarette smokers of mean age 32.22 in years (as cases). A detailed history was taken from all 100 semen donors.

### Statistical analysis

Comparison of MDA level and sperm count between smokers and non smokers was done by 'The test of significance of difference between two means'. Comparison of sperm motility between smokers and non smokers was done by 'Chi-square test'. [11, 12] Division of smoker group was done in three subgroups on the basis of smoking index. To compare these three subgroups, for sperm count and MDA level, 'Analysis of Variance' (ANOVA) test is applied.

## RESULTS

Our objectives to study the effect of cigarette smoking on MDA level, sperm count and sperm motility and to analyze the combined effects of number of cigarettes smoked per day and duration of exposure to cigarette smoking in the years (Smoking index) on MDA level and sperm count resulted as

- Smokers showed significant increase in MDA level than non smokers.
- Smokers showed significant decrease in sperm count than non smokers.
- Smokers showed significant decrease in sperm motility than non smokers.
- Smokers showed significant decrease in sperm count as smoking index is increased.
- Non significant positive correlation is found between smoking index and MDA level.

**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 | |

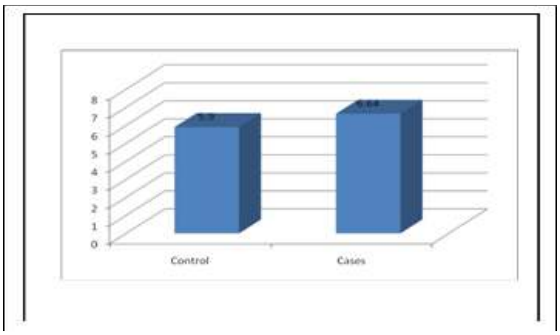



**Table 3:** Comparison of sperm count (In millions/ml) between non smokers (Controls) and smokers (Cases)

| Groups | Control | Cases |
|---|---|---|
| No. of cases | 50 | 50 |
| Mean +S.D. | 62.8+11.18 | 56.94+9.52 |
| P value | p<0.05 | |
| Significance | Significant | |

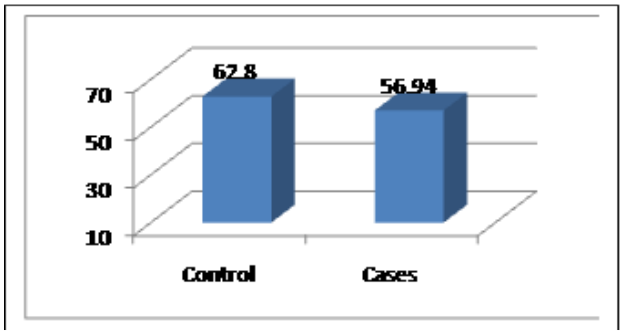
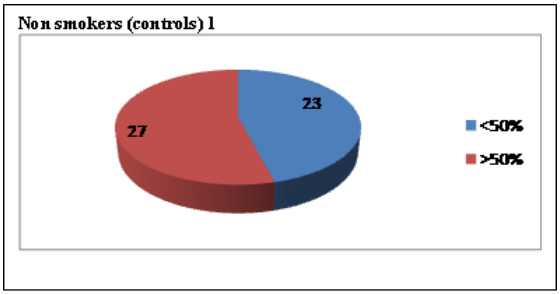



**Table 5:** Comparison of sperm motility (In percentage) between non smokers (Controls) and smokers (Cases)

| Groups | Control | Cases |
|---|---|---|
| No. of cases | 50 | 50 |
| X2 value | 9.16300 | |
| P value | p<0.001 and >0.005 | |
| Significance | Significant | |



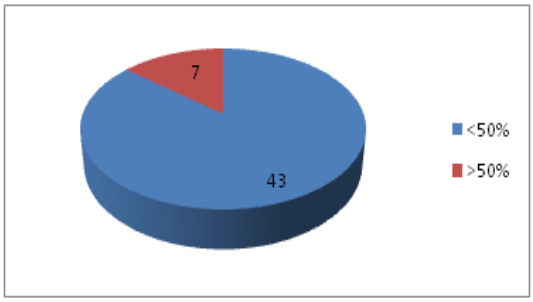



**Table 5:** Comparison of sperm count (millions/ml) among smoker group according to smoking index

| Groups | <100 | 100-200 | >200 | Total |
|---|---|---|---|---|
| No. of cases | 28 | 16 | 6 | 50 |
| Mean + S.D. | 60.21+9.61 | 54.56+6.77 | 48+8.85 | 56.94+9.52 |
| F-ratio | | F Value < 3.15 | | |
| Significance | | Non Significant | | |

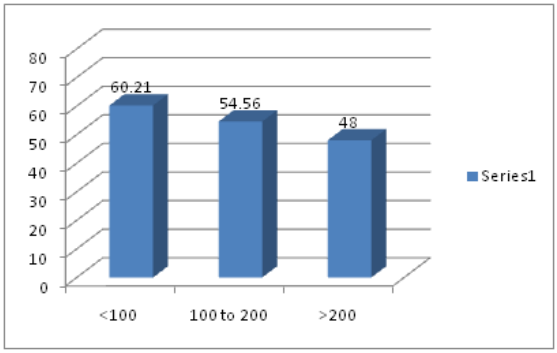
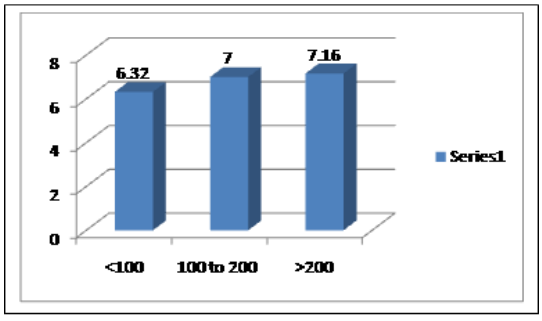



**Table 6:** Comparison of MDA level among smokers groups according to smoking index

| 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 | | |




## 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.[1]

We found statistically significant increase in MDA level in semen of cigarette smokers as compared to non smokers.( Table no. II).Our study result matches with studies done by Koul A *et al* (2001)[3], M Arabi (2004)[39].In contrast to our study, no association is found between smoking and MDA level as shown by Jozwik M *et al* (1997)[14]

It is clear that cigarette smoking results in lipid peroxidation of sperm cell membrane with increase in MDA level in semen.

Our study shows statistically significant reduction in sperm count and sperm motility in cigarette smokers as compared to nonsmokers. (Table No. III and IV) Our study result matches with the results of studies done by Shaaraway M *et al* (1982)[23]. Vytas Kaulikauskas *et al* (1985)[24], Vogt *et al* (1986)[25], Hughes E D *et al* (1996)[26], Vine M F *et al* (1997)[27], Alexander E *et al* (1998)[19], Al Bader A *et al* (1999)[20], Arabi M(2004)[13] and S.Sinclaire (2000)[21]. Non significant association between cigarette smoking and sperm count as well as sperm motility was found in studies done by Rodriguez – Rigau *et al* (1982)[8], Shen H M *et al* (1997)[9], Sergerie *et al* (2000)[3], Wong W.Y. *et al* (2000)[3], Saleh R.A.*et al*(2003)[16].

Nicotine: Detectable level of nicotine is present in gonadal tissues and fluids of cigarette smokers.[7] Nicotine stimulate adrenal medulla increasing catecholamines in blood. It results in decreased spermatogenesis and leydig cell steroidogenesis.[23]It causes double stranded DNA breaks in sperm nuclei.[13]Byproducts of nicotine also affects sperm motility.[4]

Cotinine: Detectable level of cotinine is found in gonadal tissues and fluids. Cotinine blocks spermatogenesis.[17] It also have detrimental effect on membrane function and sperm motility.[4]

PAH (Poly aromatic Hydrocarbon):It blocks spermatogenesis and makes sperm nuclei more susceptible to denaturation.[5]

OHd G (8 hydroxydeoxyguanosine):[9] is a sensitive biomarker of oxidative DNA damage that is increased in semen of cigarette smokers and reduces the sperm count and motility. Anti sperm antibodies:[9, 16] are increased in semen of cigarette smokers which reduce sperm count. CAT (Choline acetyl transferase):[4] facilitate sperm motility**.** Cigarette smoke condensates possess inhibitors of CAT. These lead to reduced sperm motility. Significantly higher levels of tail anomalies and percent

of coiled tails are found in cigarette smokers. These defective tails may be involved in abnormal sperm motility.[7]

Dietary**:** Smokers possess lower concentrations of non methyltetrahydrofolate in semen which leads to reduced sperm count.[8]

A significant positive correlation is found between smoking index and sperm count. As smoking index is increased sperm count is decreased. These findings match with studies done by vine MF *et al* (1996)[7], Wang SL *et al* (2001)[12], Taymour Mostafa (2004)[13], Vogt. H. J. (1996)[6] Non significant positive correlation is found between smoking index and MDA level. This may be because, oxidative damage in smokers is due to the number of hours of active exposure to cigarette smoke.[14]

## CONCLUSIONS

The present study was done at S.R.T.R.Medical College, Ambajogai and Government Medical College, Latur. About 100 semen samples were studied for sperm count and sperm motility. Out of these 100 samples, 50 semen samples were obtained from cigarette non smokers (as controls) and 50 semen samples were obtained from cigarette smokers.

- Smokers showed statistically significant increase in MDA level than non-smokers
- A statistically significant decrease about sperm count and motility was found in smokers as compared to nonsmokers.
- Comparison among smoker group is done on the basis of smoking index.

Statistically significant positive correlation between smoking index and Sperm count is found and statistically non significant positive correlation was found between smoking index and MDA level. Our study suggests that cigarette smoking do possess detrimental effect on sperm count and sperm motility through lipid peroxidation and other contributory factors. It is also related to duration of exposure to cigarette smoke.

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Source of Support: None Declared
Conflict of Interest: None Declared