DOI: 10.5958/j.2319-5886.2.3.078

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 2 Issue 3 July - Sep Coden: IJMRHS Copyright @2013 ISSN: 2319-5886

Received: 3rd May 2013 Revised: 30th May 2013 Accepted: 2nd Jun 2013

Research article

STUDY ON EFFECT OF CIGARETTE SMOKING ON SPERM COUNT AND SEMINAL MALONDIALDEHYDE LEVELS OF INFERTILE MEN

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ABSTRACT

The highest prevalence of smoking is observed in young adult males during their reproductive period. Smoking has been suggested to contribute to a number of diseases including male infertility. Although much is known now about the carcinogens in tobacco cigarette smoke and their resultant effects on organs like lungs and urinary bladder, their effects on fertility status have been less documented. The present study was aimed at studying the effect of cigarette smoking on sperm count and seminal MDA levels of infertile men. A total of one hundred infertile men (50 Nonsmokers and 50 Smokers) between the age group 20-45 years were taken into study. The Sperm count and seminal MDA levels in the infertile Nonsmokers and the infertile Smokers group were compared using Z Test. Infertile Smokers which were divided into Group A (≥1 and ≤10 cigarettes/ day), Group B (>10 and <20 cigarettes/ day) and Group C (≥20 cigarettes/ day) were analyzed for Sperm count and MDA levels of ANOVA Test. We observed that the sperm count was significantly lower ($p<0.01$) and the seminal MDA levels were significantly higher ($p<0.01$) in an infertile Smokers group than the infertile Nonsmokers group. We also observed that the sperm count was significantly decreased ($p<0.05$) and the seminal MDA levels were significantly increased ($p<0.05$) in accordance with the severity of smoking.

Keywords: Smokers, Non smokers, Infertile, Sperm count, Seminal MDA levels.

INTRODUCTION

The highest prevalence of smoking is observed in young adult males during their reproductive period. Smoking has been suggested to contribute to a number of diseases including male infertility. It has been reported that tobacco smoke contains some of the most deadly toxic chemicals. Smokers inhale directly and absorb the following substances: nicotine, carbon monoxide, nitrogen oxide, mutagenic pyrolysis-derived compounds and cadmium. Most of them are known to be mutagens and carcinogens, directly affecting male and female gametes and embryos.[1] Although much is known now about the carcinogens in tobacco cigarette smoke and

their resultant effects on organ like lungs and urinary bladder, their effects on fertility status have been less documented.[2] Infertility is one of the most tragic of all marital problems. Facing infertility can be very difficult for both men and women. It is emotionally stressful and physically taxing on most couples. Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse.[3] It is estimated to affect 10%–15% of all couples.[4]

Male infertility plays a key role in conception difficulties of up to 40% infertile couples.[2] The term 'male infertility' does not constitute a defined clinical syndrome but rather a collection of different conditions exhibiting a variety of etiologies and varying prognosis.[5] Although in some men a specific disorder may be present, in majority no apparent reason for infertility could be found. This has drawn attention to the impact of lifestyle and environmental factors, especially diet, obesity, smoking, alcohol intake, recreational drug use, and exposure to environmental toxins, on the reproductive health of such men. [2] The male factor, in the form of defective sperm quality, is a major cause.[5] There is a correlation between cigarette smoking in infertile men and increased leukocyte infiltration into semen .The latter has been linked to significantly increased levels of seminal oxidants i.e. Reactive Oxygen Species (ROS). Undercompromised conditions such as stress, may adversely affect reproductive health leading to infertility.[4] ROS attack polyunsaturated fatty acids in the cell membrane leading to chain of chemical reactions called Lipid Peroxidation. Malondialdehyde (MDA) is a byproduct of lipid peroxidation which represents the level of lipid peroxidation. [1]

Cigarette smoking is an avoidable lifestyle factor observed to have a negative impact on male fertility. [6,7,8,9,10,11] The aim of our study was to compare sperm count & seminal MDA levels of infertile men who were cigarette smokers with infertile non-smoking men, in order to ascertain the effect of cigarette smoking on the quality of seminal fluid.

Aims and objectives

Aim: To study the effect of cigarette smoking on Sperm count and Seminal Malondialdehyde (MDA) levels of infertile men.

Objectives: Primary:

1. To study sperm count in infertile Nonsmoker and infertile Smoker men.
2. To estimate seminal MDA levels in infertile Nonsmoker and infertile Smoker men.

Secondary:

1. To compare sperm count in infertile Nonsmoker and infertile Smoker men.
2. To compare seminal MDA levels in infertile Nonsmoker and infertile Smoker men.

MATERIAL AND METHODS

The present study was carried out in the Department of Physiology in collaboration with the Department of Biochemistry. The study protocol was approved by the Institutional Ethical Committee. Before enrollment in the study, informed written consent was obtained from each subject.

The subjects referred to semen analysis from the couples attending the outpatient department (O.P.D.) of obstetrics and gynecology (OBGY), for infertility evaluation, were enrolled in the present study.

Inclusion criteria:

Clinically infertile subjects with a history of infertility persisting longer than one year in reproductive age group were included in the study.

Primary infertile subjects were included in the study.

A total of one hundred infertile men (fifty Nonsmokers and fifty Smokers) between the age group 20-45 years were taken into study. The study was undertaken for duration of 12 months.

Nonsmokers were the men who had never smoked.

Smokers were the men who smoke cigarettes for 5 years or more and smoking till date.

The infertile Smokers were in turn divided into the following groups:

Group A (n = 28) - (≥1 and ≤10 cigarettes/ day),
Group B (n = 17) - (>10 and <20 cigarettes/ day)
Group C (n = 5) - (≥20 cigarettes/ day)

Exclusion criteria: 1. History of tobacco chewing and alcohol intake. 2. History of injury to tests, varicocele, hydrocele or undescended testes. 3. History of any chronic illness like Tuberculosis, diabetes, hypertension, thyroid disease. 4. History of UTI, occupational exposure to chemicals or excess heat. 5. Azoospermic Subjects. 6. A history of taking drugs like Vitamin E, Vitamin C or Glutathione supplementation.

Sample collection and semen analysis: Semen samples were collected by masturbation into a sterile, wide mouthed container, after at least 72 hours (3 days) of sexual abstinence. Samples were allowed to liquefy at room temperature (25ºc) for at least 45 minutes. After liquefaction, samples were analyzed for sperm count according to World Health Organization (WHO) guidelines.[12] MDA levels were estimated by using Thiobarbituric acid (TBARS) assay by Rao et al method[13]

Semen sample preparation: Liquified semen samples were centrifuged at 1200 rpm for 20 minutes for separation of plasma. This plasma used for biochemical assay. Thiobarbitutric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature 95ºc for 60 min to form thiobarbituric acid reactive substance (TBARs). The absorbance of the resultant pink product was measured Spectrometrically at 530nm The results were expressed as nmol MDA/ ml of seminal plasma.

RESULTS

In the present study, all the calculations and statistics were done using Microsoft Excel 2007 and "graph pad prism 5 software" version 5.01 was used. A 'p' value of less than 0.05 ($p < 0.05$) was considered to be statistically significant. A 'p' value of less than 0.01 ($p < 0.01$) was considered to be statistically highly significant.

For each parameter, the mean value and standard deviation were calculated. Z test was applied to study the difference between infertile Nonsmokers group and infertile Smokers group. Sperm count and MDA levels in all three groups of infertile Smokers were compared using one way ANOVA (analysis of variance) test. The observations and results of the present study were tabulated as below:

Table.1: Comparison of sperm count, MDA in infertile Nonsmokers and infertile Smokers group*

Parameter	Nonsmokers (N=50)	Smokers (N=50)	P Value
Sperm Count (millions/ejaculate)	60.88 ± 31.94	42.14 ±37.92	<0.01**
MDA (nmol/ml)	0.1597±0.0952	0.2452±0.1397	<0.01**

* Data presented as Mean ± SD, ** Significant

Table.2: Comparison of Sperm count, MDA in three groups of infertile Smokers

GROUP	N	Sperm Count (millions/ejaculate)	P Value	Seminal MDA (nmol/ml)	P Value
A	28	54.35±39.80	<0.05	0.210±0.122	<0.05
B	17	30.23±32.01		0.261±0.155	
C	5	14.2 ±14.42		0.386±0.081	

DISCUSSION

Cigarette smoking is a serious health problem of most societies. Consumption of tobacco exerts widely adverse effects on different aspects of health.

The results obtained in the present study showed that the mean ± SD of sperm count in infertile Nonsmoker men was 60.88 ± 31.94 (millions/ejaculate) and in infertile Smoker men the value was 42.14 ± 37.92 (millions/ejaculate). The intergroup comparison of the sperm count has shown that the sperm count was decreased in the infertile Smokers group. The difference of the mean sperm count in both the groups was statistically significant ($p<0.01$). The individuals with cigarette smoking thus are related to the reduced sperm count.

The sperm count decreased in accordance with severity of smoking and these values were statistically significant ($p<0.05$).

The above results of the present study are in accordance with the following studies:

Chia et al[14] reported that sperm concentration decreased due to smoking. Goverde et al[15] disclosed the sperm count difference between heavy smokers and nonsmoking men. Zhang et al[16] showed that the sperm count was negatively correlated with the amount and duration of cigarette smoking and concluded that medium heavy, and long term smoking adversely affected the semen quality. Kunzle et al[7] found a significant decrease in sperm density of smoking males, compared to non-smoking controls. Ramlau-Hansen et al[6] found a 19% lower sperm concentration in smoking men, compared to non-smokers. Also they observed a statistically dose-response relationship between current cigarette smoking and sperm concentration. Mehrannia[11] showed that sperm concentration was significantly lower in the nonsmoker men than in smoker men. Ochedalski et al[17] found that sperm count was lower in smokers when compared to nonsmokers. Olayemi[3], Mostafa[18] and Collodel et al[19] also found similar results.

The serum level of nicotine & cotinine adversely affects spermatogenesis. [20] Zinc is vital for spermatogenesis. Its deficiency leads reversibly to reduce sperm count.[21] Cigarette smoking causes significant decrease in seminal plasma zinc in smokers. Decrease in seminal plasma zinc may be associated with a decrease in antioxidant defenses which could be a contributor to the effects of cigarette smoking on sperm parameters like sperm count. Also there is a clear correlation between seminal plasma zinc levels and the extent of smoking. [22] Zn deficiency induces atrophy of seminiferous tubules and causes failure of spermatogenesis.[23]

Cigarette smoke adversely affected germ cells in testis.[24] and there is secretory deficit of Leydig and Sertoli cells on exposure to cigarette smoke.[25] Cigarette smokers were also shown to have higher levels of circulating estradiol and decreased levels of LH, follicle-stimulating hormone (FSH) and prolactin than non-smokers, all of which can negatively impact spermatogenesis.[17]

The results obtained in the present study showed that the mean ± SD concentration of seminal MDA in infertile Nonsmoker men was 0.1597 ± 0.0952 (nmol /ml) and in infertile Smoker men the value was 0.2452 ± 0.1397 (nmol /ml). The intergroup comparison of the seminal MDA levels has shown that the seminal MDA levels increased in the infertile Smokers group. The difference of the mean concentrations of MDA in both the groups was statistically significant ($p<0.01$). The individuals with cigarette smoking thus are related to the elevated MDA levels.

The seminal MDA levels increased in accordance with the severity of smoking and these values were statistically significant ($p<0.05$).

The above results of the present study are in accordance with the following studies:

Agarwal and Prabakaran[26] in their review article stated that smoking enhances ROS generation which has destructive effects on sperm DNA. Mehran[27] evaluated direct effect of seminal plasma from smokers on spermatozoa of non smokers and found impairment in membrane integrity by elevation in MDA (Malondialdehyde) levels. Kiziler et al[28] showed that MDA levels in the smoker group were significantly higher than those in nonsmoker male. Hammadeh et al[29] showed that Reactive oxygen species (ROS), Malondialdehyde (MDA), 8-Hydroxyguanosine (8-OHdG) and cotinine were significantly higher in smokers than in nonsmokers.

Smoking increases Reactive Oxygen Species (ROS) levels and decreases seminal antioxidants and ROS produced by spermatozoa is negatively correlated with the quality of sperm in semen.[30] Analysis of the fatty acid content of human spermatozoa has revealed a high degree of polyunsaturation and this factor together with the capacity of these cells to generate reactive oxygen species (ROS) render them particularly susceptible to oxidative stress. Unfortunately the spermatozoa are unable to repair the damage induced by oxidative stress because they lack the cytoplasmic enzyme systems that are required to accomplish this repair.[31,32]

Normally, a balance is maintained between the amount of ROS produced (pro-oxidants) and that scavenged by a cell (antioxidants). Cellular damage arises when this equilibrium is disturbed, especially when the cellular scavenging systems cannot eliminate the increase in ROS[33]. ROS attack Polyunsaturated Fatty Acid (PUFA) in the cell membrane leading to a chain of chemical reactions called lipid peroxidation. Malondialdehyde (MDA) is a byproduct of lipid peroxidation which represents the level of lipid peroxidation. [1]

The presence of tobacco smoke constituents in seminal plasma could provide a warning of the adverse effects of cigarette smoke on the physiology of reproduction. The clinical significance of present finding should be to develop effective interventions aimed at helping patients stop smoking for the benefits to the general health and for their fertility. Hence we suggest that every smoker should be encouraged to stop smoking especially if pregnancy is planned.

CONCLUSION

It is concluded that cigarette smoking adversely affects Sperm count and seminal MDA levels and in turn semen quality. Deterioration in semen quality appears in direct proportion to the number of cigarettes smoked.

ACKNOWLEDGEMENT

We acknowledge this work to all the staff of Department of Physiology, Government Medical College, Aurangabad who directly or indirectly helped us to get this work completed.

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