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Cigarette Smoking and the Risk of Male Infertility

[1]A. Hosseinzadeh Colagar, [2]G.A. Jorsaraee and [1]E. Tahmasbpour Marzony
[1]Department of Biology, Faculty of Basic Science, Mazandaran University, Babolsar, Iran
[2]Department of Fertility and Infertility, Fateme Zahra Hospital,
Babol University of Medical Sciences, Babol, Iran

Abstract: In this research we investigated the effect of cigarette smoking on sperm parameters both before and after swim-up. Semen sample provided from fertile smoker (n = 25), fertile nonsmoker (n = 21), infertile smoker (n = 23) and infertile nonsmoker men (n = 32). Semen analysis was performed manually according to the World Health Organization (WHO) standards guidelines. Present research showed that sperm parameters quality in smoker men was approximately lower than nonsmoker men. As well as present research showed that cigarette smoking has dose dependent effect on sperm parameters, but this effect was not significant. Therefore, it appears that cigarette smoking is associated with reduced sperm quality and the risk of idiopathic male infertility in smoker men.

Key words: Sperm parameters, male infertility, cigarette smoking, smoker and nonsmoker men

INTRODUCTION

Although cigarette smoking is a widely recognized health hazard and a major cause of mortality (ASRM, 2004 and 2006), people continue to consume cigarettes on a regular basis. According to the World Health Organization (WHO, 1997-1999), approximately one third of the world's population older than 15 years, are smoker (Saleh *et al*., 2002; Kunzle *et al*., 2003). The highest prevalence of smoking is observed in young adult males during their reproductive period (46% smokers between 20 and 39 years) (Langgassner, 1999). An additional portion of nonsmokers, especially children, are also affected as second hand smokers by inhaling side stream smoke from burning cigarettes and exhaled smoke from smokers (Zavos, 1999; Saleh *et al*., 2002). Evidence indicated that cigarette smoking has become a serious health and societal problem today and also presents a rather challenging dilemma for the physician or the health care provider. It can be said today that smoking has been established as the number one preventable cause of death and disease in the countries worldwide (Zavos *et al*., 1999). Most lung cancer and emphysema, as well as a high percentage of heart attacks are caused by cigarette smoking. About 30-40% of all deaths from cancer are associated with smoking and chewing tobacco (ASRM, 2006; Wynder and Hoffman, 1994; Lee *et al*., 1998). Recent scientific data reveal that the cancer risk from smoking is not limited to cancer of the lung; it can be associated with an increased incidence of cancer in the lungs, larynx, cervix, oral cavity, esophagus, bladder and even leukemia (Lee *et al*., 1998), many bladder, cervical, esophageal and pancreatic cancers are also caused by smoking (Zavos *et al*., 1999). Cigarette smoke contains a large number of substances, including nicotine, carbon monoxide and recognized carcinogens and mutagens such as radioactive polonium, benzopyrene, dimethylbenzanthracene, dimethylnitrosamine, naphthalene and methnapthalene (Zavos *et al*., 1998; Wong *et al*., 2000; Lee *et al*., 1998). Many of these constituents, however, have never been evaluated for toxicity and therefore the complete contents of cigarettes and cigarette smoke remain unknown. Inhalation of cigarette smoke, whether through active or passive smoking, leads to absorption of these substances through the pulmonary vasculature and blood-borne circulation throughout the body (Zavos *et al*., 1998). It is also possible that those same substances could end up in the seminal plasma of smokers via various modes of diffusion and active transport (Zavos *et al*., 1998). Therefore, this is not surprising that cigarette smoking has negative effects on male reproductive system same as other tissue. But the relationship between cigarette smoking and male fertility remains controversial. Although the effect of smoking on male fertility remains inconclusive, the evidence of adverse effects of smoking on semen parameters suggest that smoking reasonably may be regarded as an infertility risk factor, smoking should therefore be discouraged for both male and female partners in couples with a history of infertility or recurrent

Corresponding Author: Dr. Abasalt Hosseinzadeh Colagar, Department of Biology, Faculty of Basic Science, Mazandaran University, Code Post: 47416-1467 Babolsar, Iran Tel/Fax: +98-112-5242022

pregnancy loss, particularly when marginal or frankly abnormal semen parameters have been documented (ASRM, 2006). Thus, the aim of this study is to evaluate the relationship between cigarette smoking and sperm quality in male partners of fertile and infertile couples undergoing infertility evaluation.

MATERIALS AND METHODS

Semen population: All samples provided from Fateme Zahra IVF center, then undergoing evaluation for infertility. Study population included fertile nonsmokers (n = 21), fertile smoker (n = 25), infertile nonsmoker (n = 32) and infertile smoker men (n = 23). Fertile and infertile smoker men grouped according to the frequency of their cigarette smoking habit to: group I (fertile smoker with 1-7 cigarettes/day, n = 14), group II (fertile smoker with >7 cigarettes/day, n = 11), group III (infertile smoker men with 1-7 cigarettes/day, n = 11) and group IV (infertile smoker men with >7 cigarettes/day, n = 12).

Semen collection and analysis: Semen samples were collected by masturbation into a sterile container after sexual abstinence for 2-3 days. All samples provided according to the WHO criteria and analyzed for appearance, volume, consistency and pH. Before semen analysis, a questionnaire was distributed to obtain information on age and lifestyle male including: smoking habits, alcohol use, use or abuse of other substances and drugs and history of orchitis, testicular trauma, sexually transmitted disease, varicocele, inguinal hernia operation, cryptorchism and etc. All samples provided from smoker and nonsmoker men and had been exception from other case. On microscopic examination, sperm concentrations, percentage of motile sperm and sperm with normal morphology were objectively evaluated. Sperm count and percentage of motile sperm evaluated according to the world health organization (WHO, 1997-1999), whereas percentage of sperm morphology evaluated according to Kruger's criteria (Kruger *et al*., 1986). An aliquot of semen samples washed (swim-up) in Hams F10 medium (include 10% BSA, bovine serum albumin) for half hours in 37°C and then used for sperm count, motility and morphology evaluation.

Statistical analysis: Mean standard (mean±SD) of sperm parameters quality in the smoker and nonsmoker men analyzed by descriptive statistic. The relationships of sperm count, motility and morphology between fertile smoker-nonsmoker and infertile smoker-nonsmoker men were compared with Independent Samples t-test model. A p-value <0.05 was considered statistically significant.

RESULTS

The initial study sample consisted of 101 participants, of whom 20.79% (21/101) were fertile nonsmoker, 31.61% (32/101) were infertile nonsmoker, 24.75% (25/101) were fertile smoker and 22.77% (23/101) were infertile smoker men. Sperm parameters quality between fertile smoker, nonsmoker and infertile smoker, nonsmoker men evaluated both before and after swim-up and showed in Table 1 and 2, respectively. There was no significantly difference between age of fertile smoker, nonsmoker (p-value = 0.351) and infertile smoker, nonsmoker men (p-value = 0.231).

Sperm parameters quality before swim-up: Present results showed that sperm parameters in smoker men were approximately lower than nonsmoker men. The statistical analysis between fertile smoker-nonsmoker men showed that sperm counts, motility and morphology of the smoker men are lower than nonsmoker men (Table 1). Sperm count and morphology in fertile smoker men were also strongly affected (p-value = 0.03 and 0.05, respectively), whereas the percentage of sperm motility was slightly but not significantly reduced (p-value = 0.891). Ejaculate volume was not different between fertile smoker-nonsmoker men. In addition statistical analysis in infertile smoker-nonsmoker showed that sperm quality in infertile smoker is lower than infertile nonsmoker. Sperm count and morphology in infertile smoker were significantly lower than nonsmoker men (p-value = 0.05 and 0.03, respectively), whereas sperm motility in infertile smoker men was also slightly lower than infertile nonsmoker men (p-value = 0.935). Semen volume was not significant different in both infertile smoker, nonsmoker men (Table 1).

Sperm parameters quality after swim-up: Table 2 shows the sperm count and the percentage of sperm morphology, motility in all samples after swim-up. Statistical analysis in fertile smoker-nonsmoker showed that sperm morphology in fertile smoker men is significantly lower than fertile nonsmoker men (p-value<0.01), whereas sperm count and motility in fertile smoker men were slightly lower than fertile nonsmoker men (p-value = 0.141 and 0.314, respectively). Sperm morphology in infertile smoker men was significantly lower than infertile nonsmoker men (p-value = 0.04), whereas sperm count and motility were slightly lower (p-value = 0.39 and 0.07, respectively).

Table 1: Sperm parameters quality in fertile and infertile nonsmoker and smoker men, before swim-up

	Nonsmoker men (n = 53)		Smoker men (n = 48)	
Sperm parameters	Fertile (n = 21)	Infertile (n = 32)	Fertile (n = 25)	Infertile (n = 23)
Age (years)	31.38±4.36	29.55±4.46	29.7±5.14	31.37±7.4
Volume (mL)	4.14±1.36	3.85±1.53	4.35±1.47	3.00±1.53
Sperm count ($\times 10^6$ mL)	80.00±29.63**	36.90±29.91*	71.0±28.81**	31.81±21*
Total sperm ($\times 10^6$)	330.71±145.60	137.11±124.33	310.5±150.63	93.26-±74.04
Motility (%)	73.10±16.3	50.06±29.69	72.0±16.73	43.75±32.99
Normal morphology (%) (by Kruger's strict criteria, 1986)	14.93±3.63	5.96±4.36**	12.9±4.78	3.75±2.11**

Results are presented as mean±SD; *p = 0.05, **p<0.05

Table 2: Sperm count and motility in fertile and infertile nonsmoker and smoker men, after swim-up

	Nonsmoker men (n = 53)		Smoker men (n = 48)	
Sperm parameters	Fertile (n = 21)	Infertile (n = 32)	Fertile (n = 25)	Infertile (n = 23)
Sperm count ($\times 10^6$ mL)	67.62±27.58	28.96±23.1	55.27±27.58	23.00±17.6
Motility (%)	88.57±35.86	55.97±34.16	79.28±36.83	36.32±30.41
Normal morphology (%) (by Kruger's strict criteria)	16.29±5.33*	6.85±4.22**	13.30±4.91*	4.38±1.96**

Results are presented as mean±SD; * p<0.01, **p<0.05

Table 3: Sperm parameters quality in group I, II, III and IV

	Fertile (n = 25)		Infertile (n = 23)	
Sperm parameters	Group I (n = 14)	Group II (n = 11)	Group III (n = 11)	Group IV (n = 12)
Cigarette dosing	3.87±2.35	12.33±4.51	3.67±2.75	12.00±5.57
Volume (mL)	4.14±1.59	4.75±1.03	3.12±1.58	3.10±1.14
Sperm count (10^6 mL)	73.57±28.44	65.00±31.46	30.83±18.51	29.00±23.89
Motility (%)	72.66±16.61	65.83±19.6	51.42±31.97	47.00±30.38
Morphology (%) (by Kruger's strict criteria)	13.80±3.85*	9.50±6.22*	4.20±1.18	3.16±2.31

Results are presented as mean±SD; *p-value<0.05. Group I: Fertile smoker men that Smoke 1-7 cigarettes per day, Group II: Fertile smoker men that smokes >7 cigarettes per day, Group III: Infertile smoker men that smokes 1-7 cigarettes per day and Group II: Infertile smoker men that smokes >7 cigarettes per day

Sperm quality in smoker groups: Fertile-infertile smoker men divided to four groups (groups 1-4) according to cigarettes habitat (Table 3). Present results shows that sperm morphology in group-2 were also strongly affected and were lower than group-1 (p-value<0.05), whereas sperm count and motility slightly affected. In addition statistical between groups III and IV showed that sperm parameters quality in group III was slightly lower than group IV (Table 2), but these difference was not significantly. Therefore, present study showed that (i) cigarette smoking cause to low sperm quality in smoker men and (ii) has a dose-dependent effect on sperm quality.

DISCUSSION

We investigated sperm parameters quality both before and after swim-up in smoker and nonsmoker men. Present study showed that cigarette smoking has negative affects on sperm count, motility and normal morphology. These results were consistent with other studies (Kunzle *et al*., 2003; Saleh *et al*., 2002; Stillman *et al*., 1986; Vine *et al*., 1996; Close *et al*., 1990; Sofikitis *et al*., 1995). These studies have shown that cigarette smoking affects on sperm concentration, motility and morphology and related with poor sperm quality. On the other hand, present results contradict the findings of other studies that have found no association between smoking and sperm quality or sperm function (Dikshit *et al*., 1987; Vogt *et al*., 1986). Although these studies showed the negative effects on sperm parameters quality, but the mechanisms by which smoke affects spermatozoa are poorly understood (Kunzle *et al*., 2003; Saleh *et al*., 2002). Recently, studies show that cigarette smoking cause to low semen quality with several mechanisms. One of these mechanisms is seminal oxidative stress induced ROS, which has destructive effects on sperm quality and function. Saleh *et al*. (2002) show that oxidative stress status in semen of smoker men is significantly higher than nonsmoker men. Studies show that cigarette smoking lead to increase of seminal oxidative stress with several mechanisms: (i) cigarette smoke itself contains high levels of ROS such as superoxide anion (O_2), hydrogen peroxide ($H O_2$) $_2$and hydroxyl radicals (OH) (Saleh *et al*., 2002; Lee *et al*., 1998; Kunzle *et al*., 2003; Church and Pryor, 1990; Pryor and Stone, 1993), (ii) Smoking metabolites may induce an inflammatory reaction in the male genital tract with a subsequent release of chemical mediators of inflammation that can recruit and activate leukocytes. Activated leukocytes can generate high levels of ROS in semen, which may overwhelm the antioxidant strategies, resulting in oxidative stress (Saleh *et al*., 2002) and (iii) Toxic metabolites of cigarette smoke may impair

spermatogenesis, resulting in the production of abnormal spermatozoa, that is a important source of ROS and oxidative stress (Saleh *et al*., 2002; Karagounis *et al*., 1985). ROS produced by cigarette smoke-induced phagocyte cells or abnormal spermatozoa cause oxidative damage to normal sperm DNA, protein and lipids, which may be closely related to sperm dysfunction (Aitken and Baker, 2006). Spermatozoa are particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) and their cytoplasm contains low concentrations of scavenging enzymes (Agarwal *et al*., 2002; Agarwal and Prabakaran, 2005; Aitken and Baker, 2006). Therefore, production of high levels of ROS in the reproductive tract is detrimental not only to the fluidity and function of the sperm plasma membrane but also to the integrity of DNA in the sperm nucleus (Agarwal and Saleh, 2002; Agarwal and Prabakaran, 2005). DNA damage included by excessive levels of ROS may accelerate the process of germ cell apoptosis, leading to decline in sperm counts and associated with male infertility (Agarwal and Allamaneni 2004; Aitken and Krausz, 2001). Studies found that levels of ROS correlate with motility of spermatozoa (Iwasaki and Cagnon, 1992; Aitken *et al*., 1989; Agarwal *et al*., 1994; Armstrong *et al*., 1999). Peroxidative damage to the sperm membrane and axonemal proteins appears to be the cause of permanent impairment in sperm motility (Agarwal and Allamaneni, 2004). By accomplished research (Close *et al*., 1990; Dikshit *et al*., 1987; Kunzle *et al*., 2003; Said *et al*., 2005; Saleh *et al*., 2002; Guzick *et al*., 2001; Martini *et al*., 2004; Pasqualotto *et al*., 2004; Wang *et al*., 2001), it appears that negative effects of cigarette smoking on sperm parameter may have a dose-dependent effect and high level of cigarette smoking is positive related with decreased sperm parameter quality. This is may be do to cigarette toxic components that cause to increase of seminal free radicals and oxidative stress (Karagounis *et al*., 1985). Oxidative stress has a destructive effect on sperm membrane and DNA and associated with low sperm quality in smoker men.

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