The Impact of Opium Dependence and Cigarette Smoking on Human Semen Parameters

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A B S T R A C T

Introduction: To examine the effects of opium dependence and cigarette smoking on semen quality and sperm parameters, the semen quality of men who abuse opiates and/or smoke cigarettes was investigated in a retrospective study.

Methods: Male partners of 1325 infertile couples attending the infertility clinic for intrauterine insemination (IUI) procedure were divided into non-smoking non-opium (NS/NO), smoking and non-opium (S/NO), non-smoking and opium (NS/O), smoking and opium (S/O), and opium dependence regardless of smoking status (O/RS) groups. Two samples were collected from each subject and were analyzed in accordance to WHO criteria.

Results: Subjects in different groups were comparable regarding mean age, duration of abstinence, and familial history of infertility, whereas duration of infertility was longer in all groups than in NS/NO group (P<0.05). The volume of semen, liquefaction time and pH differed significantly between S/NO and NS/NO groups (P<0.05). In addition, more men in S/NO group were diagnosed to be teratozoospermic than other groups (P=0.018). Sperm progression was significantly lower in NS/O than in NS/NO group (P<0.05).

Conclusion: These findings suggest that opium dependence and cigarette smoking alter semen and sperm production and quality differently.

Key Words:
Opium dependence, Cigarette smoking, Semen analysis

1. Introduction

Despite the worldwide antismoking campaign, cigarette smoking is very common in various societies. The highest prevalence of smoking is observed in young men during their reproductive period [1]. About a third of the world’s population aged 15 and older daily smoke cigarettes [2]. Since cigarette smoke is known to contain mutagens and carcinogens [3], there has been much concern that smoking may be associated with subfertility in males with some reports of decreased sperm concentration, lower sperm...
motility, and a reduced percentage of morphologically normal sperm [4-6]. Cigarette smoking has also been correlated with poor sperm function in sperm penetration assays [7]. Smokers commonly have a significantly higher rate of spermatozoa with DNA fragmentation than non-smokers [8]. In addition, tobacco smoking as a source of reactive oxygen species, can cause an increased oxidative DNA damage in leukocytes [9] and smoking may result in subfertility by influencing hormone levels [10]. Estradiol levels are mainly found to be elevated although testosterone levels may be unchanged, elevated or decreased in smokers [7]. Significant decrease of semen volume has also been reported in some studies [3, 11, 12], whereas few studies found no meaningful differences [13].

Semen analysis is regarded as a valid approach for assessing male reproduction quality. In a meta-analysis of 27 studies on cigarette smoking and semen quality, a mean reduction of 13% in sperm concentration, 10% in sperm motility, and 3% in normal sperm morphology was reported in smokers [7]. In that meta-analysis, the number of subjects was lower than 200 men in 25 out of 27 studies. Although, most studies have shown a negative correlation between cigarette smoking and sperm parameters [4, 6, 14], some have found no correlation between cigarette smoking and semen quality [1, 9, 10, 15].

In contrast to worldwide studies on the impacts of cigarette smoking on male subfertility, very few studies have addressed the impact of opiate dependence on the reproductive system. Opiates and cocaine are considered to be the main drugs of abuse worldwide [16]. In the United States, 2–3 million people are currently cocaine users and an estimated 119,000 abuse heroin [16]. A recent study conducted in Kerman province in southeast Iran, has shown that 11.5% of the male individuals abuse opiates [17]. Opiates and cocaine have been shown to exert important physiologic effects on multiple organ systems including the reproductive system [16]. These drugs may interfere with hypothalamic activity and also directly affect testosterone synthesis [18]. Opiate dependence is proposed to be associated with abnormal spermatogenesis in men [19], and has been correlated with reduced sexual performance as well [16].

The relationship between cigarette smoking, semen parameters, and the fertility status remains a controversial issue. In addition, consequence of drug abuse, such as opium dependence on semen parameters, constitutes an area that requires further researches. Compared to the other drugs of abuse, opium use is a relatively common life style in Iranian society, especially among men [20]. Thus, the aim of this study was to compare the various semen and sperm characteristics of the male partners of infertile couples with regard to their smoking and opium dependence status. These couples were referred to the Infertility Research and Clinic Center in the Kerman University of Medical Sciences.

2. Materials & Methods

Subjects

An Institutional Review Board approval was obtained for the study from Kerman University of Medical science ethics committee, Kerman, Iran. The data obtained from ejaculate samples of male partners of 1325 infertile couples attending the University-based infertility clinic for intrauterine insemination (IUI) procedure between December 2002 and November 2008 were analyzed retrospectively. Most of the subjects were referred by gynecologists, urologists or were self-referred. The demographic characteristics as well as smoking and opium dependence history was obtained using a self-reported questionnaire and a direct interview wherever needed. An urologist who was a specialist in male infertility evaluated subjects as a routine pretreatment procedure for IUI and those eligible for IUI [21] were enrolled in this study. Men with a history of genital abnormalities, severe and moderate varicocele, occupational exposure to chemicals or excessive heat, for example, cases working at chemical factories, petrol pumps, and bakeries, and azoospermic men were excluded.

Subject categorization and sample collection

An informed consent letter was taken as a routine procedure from all subjects. At least two semen samples were collected with an interval of one month. The semen analysis reports taken at the time of IUI procedure were included in the study. The subjects were categorized into four groups as non-smoking and non-opium (NS/NO; the control group) who had no history of smoking and opium dependence, smoking and non-opium (S/NO) group; men smoking ≥10 cigarette every day for at least 6 months and with no history of opium dependence, non-smoking and opium (NS/O) group; men with a history of ≥1 year dependence on regular daily opium consumption (smoking opiate) without a cigarette smoking history, smoking and opium (S/O) group; men who were simultaneously both opium dependent and a smoker, and finally a group was created from calculation of S/O and NS/O groups with a history of opium dependence regardless of smoking status (O/RS).

All subjects were asked to provide their semen sample in the laboratory after sexual abstinence of 2–7 days and...
to collect their semen sample in a clean container by masturbation. Few individuals who refused or were unable to provide semen sample by masturbation, provided their semen sample after intercourse with their partner. Samples were immediately transferred to the laboratory and standard clinical semen examination was performed as soon as the samples were liquefied at 37°C according to WHO criteria [22].

**Semen analysis**

Following semen and sperm characteristics were noted in this study: liquefaction time, volume, viscosity (for ease of statistical analysis, viscosity was rated on a 1–4 scale; 1 for thinnest and 4 for thickest specimens), pH, concentration, motility, sperm forward progression, percentage of viable sperms after eosin exclusion dye staining, number of round cells and WBCs per milliliter×10⁶, and percentage of sperms with normal morphology. Morphology was assessed after modified papanicolao staining of samples. In each slide, 200 sperm were evaluated according to WHO criteria.

**Semen analysis classification**

The results of semen analyses were classified according to the nomenclature of semen variables (World Health Organization, 1993). Normozoospermia was identified when morphology and sperm concentration were within the reference values. The value for ‘sperm concentration’ was 20×10⁶ sperm/ml, for ‘motility’ ≥50% sperm with forward progression (categories ‘a’ and ‘b’) or ≥25% sperm with category ‘a’ movement, and for ‘morphology’ ≥30% sperm with normal morphology, respectively. Subjects were categorized as “Oligozoospermic” when sperm concentration was less than the value. Likewise, subjects with motility less than the normal value, and morphology less than the normal value were categorized as asthenozoospermic and teratozoospermic, respectively. Subjects with a disturbed three variables (concentration, motility, morphology) were categorized as oligoasthenoteratozoospermic.

Combinations (oligoasthenozoospermia, oligoteratozoospermia and asthenoteratozoospermia) were used when two variables were disturbed. Strict quality control measures were enforced routinely in the laboratory. Two technologists who were well-trained in semen analysis were responsible for most of the laboratory investigations. Semen samples of known sperm parameters were used for quality assessment regularly. The coefficient of variations of semen parameters, such as motility, viability, progression, sperm density, and total sperm count between the two technologists was generally less than 10%. An experienced embryologist evaluated sperm morphology according to WHO criteria.

**Statistical analysis**

Data were anonymously analyzed with SPSS 11.5 software for Windows. A descriptive analysis of the data was performed and the variables were further analyzed with analysis of variance (ANOVA) followed by Fisher’s LSD test, or with the Kruskal-Wallis test and Mann-Whitney U test depending on the normality assumption. For different categories of sperms, abstinence time, and familial history of infertility, Chi-square test or Fisher exact test was used to run a pairwise comparison between S/NO, NS/O, S/O, and O/RS groups and NS/NO group as control after Bonferroni adjustment for P. A P-value of <0.05 was considered statistically significant.

3. Results

A total of 1325 subjects were recruited for this study. Of these, 1002 (73%) were in NS/NO group, 223 (16.3%) in S/NO group, 21 (1.5%) in NS/O group, 79 (5.8%) in S/O group. In addition, 100 subjects were placed in O/RS group. Characteristics of the study subjects are reviewed in Table 1.

Subjects in control group had lower duration of infertility compared to S/NO, S/O, and O/RS groups. Besides, men in the NS/NO and S/NO groups had observed the abstinence time suggested by the urologist more accurately. None of the groups had a significantly different familial history of infertility (Table 1).

Table 2 shows the semen and sperm parameters evaluated with respect to smoking status and opium dependence. Ejaculate volume was significantly lower in S/NO, S/O, and O/RS groups compared to NS/NO group (P<0.05) and liquefaction time was significantly (P<0.01) lower in S/NO compared to NS/NO group. pH in S/NO, S/O, and O/RS groups was significantly higher than NS/NO and NS/O groups (P<0.01). Sperm concentration, the numbers of viable sperm, and the proportion of motile sperm were nearly identical among various groups with no significant difference between NS/NO group and the others. Sperm progression significantly reduced in all opium-dependent groups (S/O, NS/O, and O/RS, the level of significance ≤0.05). The numbers of morphologically normal sperm decreased significantly (P<0.001) in the S/NO group and no meaningful difference was observed between the other groups. No significant increase in the number of round cells in S/NO, S/O, and O/RS groups were detected. However, a significant decrease in NS/O group was observed compared
to NS/NO group (P<0.01). WBC count in S/O group was higher than WHO criteria, and it was significantly higher than NS/NO group (P<0.001).

Although differences were found between groups in terms of various categories of sperm (asthenozoospermia, oligozoospermia, teratozoospermia, asthenozoospermia, oligoasthenozoospermia, oligoteratozoospermia and oligoasthenoteratozoospermia), no significant differences were found between groups, except for teratozoospermia that was significantly (P=0.018) higher in S/NO group compared with NS/NO group (Table 3).

4. Discussion

Although there is a common belief on carcinogens in tobacco cigarette smoke and their possible effects on various organs including lungs and urinary bladder, their effects on male reproductive system have not been well documented [23]. In addition to cigarette smoking, the abuse of drugs such as opiates has become an ever-increasing problem among the youth especially of reproductive age. To the best of the authors’ knowledge, only few studies have been conducted to evaluate the possible effects resulting from consumption of such drugs on semen and sperm parameters and fewer studies assessing the probable relationship between opium consumption and semen parameters have been carried out. There is an increasing concern about the potential of substances in cigarette smoke and opiates to disrupt fertility by influencing semen quality as a valid approach for assessing reproduction.

In this study, retrospectively evaluating the impact of cigarette smoking and opium dependence on male partners of infertile couples attending a University-based clinic, a significant difference was found between S/NO and NS/NO groups in some semen parameters namely volume of semen, pH, liquefaction time, and sperm morphology. In addition, opium dependence was associated with few semen parameters such as decrease in progression and increase in the number of round cells. The source population differs among the different studies. Marinelli and et al. showed that most of the studies (13/21) recruited subjects from infertility centers [24], as we did in our study. We included the subjects who were suitable for IUI in this study. A relatively identical proportion of subjects in different groups was normozoosperm (48%-61.9%; Table 3). However, a control group from normal population may have shown any deviation from the norm.

In this study, the number of WBC was significantly (S/O and O/RS groups) and not significantly (S/NO and NS/O groups) higher compared to the NS/NO group.

Table 1. Characteristics of study subjects according to smoking status and opium dependence.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NS/NO</th>
<th>S/NO</th>
<th>NS/O</th>
<th>S/O</th>
<th>O/RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases (%)</td>
<td>1002 (75.6)</td>
<td>223 (16.8)</td>
<td>21(1.6)</td>
<td>79 (6)</td>
<td>100</td>
</tr>
<tr>
<td>Male age (y)</td>
<td>32.0±6.0</td>
<td>32.6±5.0</td>
<td>32.4±8.1</td>
<td>33.4±4.9</td>
<td>33.1±5.8</td>
</tr>
<tr>
<td>Duration of infertility (y)</td>
<td>4.6±3.0</td>
<td>5.9±3.6**</td>
<td>5.8±2.8*</td>
<td>6.8±4.5**</td>
<td>6.4±4.2**</td>
</tr>
<tr>
<td>≥2≤7 day abstinence, n (%)</td>
<td>746 (70.5)</td>
<td>170 (16.1)</td>
<td>15 (1.4)</td>
<td>56 (5.3)</td>
<td>71 (6.7)</td>
</tr>
<tr>
<td>&lt;2&gt;7 day abstinence, n (%)</td>
<td>256 (69.7)</td>
<td>53 (14.4)</td>
<td>6 (1.6)</td>
<td>23 (6.3)</td>
<td>29 (7.9)</td>
</tr>
<tr>
<td>Subjects with familial history of infertility, n (%)</td>
<td>70 (7)</td>
<td>17 (7.6)</td>
<td>2 (9.5)</td>
<td>9 (11.4)</td>
<td>11 (11)</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation of mean. NS/NO, non-smoking and non-opium; S/NO, smoking and non-opium; NS/O, non-smoking and opium; S/O, smoking and opium and O/RS, Opium dependence regardless of smoking status. *, P<0.05; **, P<0.001 compared to NS/NO group.
is in agreement with some studies published earlier [1, 27, 28]. Semen pH was also found to be significantly higher in S/NO, S/O, and O/RS groups. Leukocytes are the major source of reactive oxygen species (ROS) in the ejaculate [29], which may cause a 107% increase in ROS levels in seminal leukocyte concentration because of smoking [28]. On the other hand, the elevated leukocyte count as well as the increased semen pH was considered to be an indicator of inflammation [30]. Consequently, smoking may increase the number of WBC, known as an important source of ROS, and in turn, the semen pH increased through the probable inflammation phenomena.

A borderline significant increase in the number of round cells in S/NO group and a statistically significant decrease in NS/O group indicate a correlation between opium consumption and spermatogenesis and or spermiogenesis. A study on lizards has shown beta-endorphines may regulate androgen release in testis through inhibition of gonadotrophin secretion [31]. Also, proenkephalin gene expression in rat testis has shown to regulate spermatocyte differentiation during meiosis [32]. In addition, acute or chronic administration of met-enkephaline (endogenous opioid) in rat resulted in numeric and morphologic changes in Leydig cells as well as blockage in spermatogenesis that led to a fall in the number of spermatooza and secondary spermatocytes [33]. In NS/O group the significant lower number of round cells and non-significant decrease in sperm concentration may be related to opium consumption in this group.

S/NO was the only group in which the number of morphologically normal sperm decreased significantly and this reduction was reflected in the sperm categorization by which more subjects fell into terathozoospermia category. Decrease in sperm morphology according to smoking habits was also reported by some authors [5, 34-36]. However, these studies are in contrast with other studies that reported no significant difference between smokers and non-smokers [11, 37]. Testosterone is a key hormone in the spermato genesis and spermiogenesis processes. The level of steroid hormones, especially testosterone has been reported to be altered in smokers [1, 10]. This finding has also been confirmed by animal studies [38] in which a reduction in the level of testosterone was reported following a decrease in the number of rat Leydig cells in the testes of rats exposed to cigarette smoke. Change in the level of testosterone in S/NO and not in other groups may have probably affected the sperm morphology. In addition, direct effects of some known; cotinine, [9, 39] and unknown toxic cigarette components may have interfered with spermatogenesis and lead to lower rate of sperm morphology in the S/NO group. Morphology did not change significantly in NS/O as well as S/O and O/RS groups. However, a slight non-significant decrease was detected when data from the men with opium dependence and smoking habits (S/O and O/RS) was

Table 2. Mean values of the semen and sperm parameters according to the opium dependence and smoking status.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal range/unit</th>
<th>NS/NO (n=1002)</th>
<th>S/NO (n=223)</th>
<th>NS/O (n=21)</th>
<th>S/O (n=79)</th>
<th>O/RS (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>(&gt;2 ml)</td>
<td>4.13±2.1</td>
<td>3.73±1.7a</td>
<td>3.36±1.8</td>
<td>3.52±1.7b</td>
<td>3.47±1.7p</td>
</tr>
<tr>
<td>Liquefation time (&lt;30 min)</td>
<td>31.3±16.1</td>
<td>26.8±13.3c</td>
<td>36.4±19.3</td>
<td>31.5±13.7</td>
<td>32.6±15.1</td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td>(1-3)</td>
<td>2.70±0.05</td>
<td>2.75±0.12</td>
<td>2.77±0.29</td>
<td>2.85±0.16</td>
<td>2.80±0.15</td>
</tr>
<tr>
<td>pH</td>
<td>6.2-8</td>
<td>7.45±0.32</td>
<td>7.54±0.33a</td>
<td>7.49±0.22</td>
<td>7.61±0.28b</td>
<td>7.59±0.27c</td>
</tr>
<tr>
<td>Round cell</td>
<td>(&lt;2×10⁶/ml)</td>
<td>1.97±2.84</td>
<td>2.77±4.64a</td>
<td>1.19±2.62a</td>
<td>2.79±3.81</td>
<td>2.38±3.56</td>
</tr>
<tr>
<td>WBC</td>
<td>(&lt;1×10⁶/ml)</td>
<td>0.22±1.29</td>
<td>0.39±1.36</td>
<td>0.05±0.05a</td>
<td>1.33±0.73a</td>
<td>0.58±1.98a</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>(≥20×10⁶/ml)</td>
<td>77.9±52.0</td>
<td>73.7±52.4</td>
<td>71.8±45.2</td>
<td>81.4±58.3</td>
<td>78.3±55.2</td>
</tr>
<tr>
<td>Grade ‘a’ Motility (%)</td>
<td>(≥25%)</td>
<td>31.4±7.4</td>
<td>30.5±8.1</td>
<td>21.1±7.7b</td>
<td>23.9±6.2a</td>
<td>22.7±6.5a</td>
</tr>
<tr>
<td>Grade ‘a’ and ‘b’ Motility (%)</td>
<td>(≥50%)</td>
<td>59.7±15.7</td>
<td>58.6±16.4</td>
<td>48.7±17.9b</td>
<td>51.1±15.7b</td>
<td>50.6±16.0a</td>
</tr>
<tr>
<td>Viability</td>
<td>(≥50%)</td>
<td>77.3±11.5</td>
<td>76.9±11.9</td>
<td>74.3±13.0</td>
<td>75.5±11.7</td>
<td>75.3±12.0</td>
</tr>
<tr>
<td>Morphologically normal</td>
<td>(≥30%)</td>
<td>42.6±15.7</td>
<td>38.4±16.6b</td>
<td>43.0±12.2</td>
<td>41.3±13.9</td>
<td>41.2±14.1</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation of mean. NS/NO, non-smoking and non-opium; S/NO, smoking and non-opium; NS/O, non-smoking and opium; S/O, smoking and opium and O/RS, opium-dependence regardless of smoking status.

a. significantly different from NS/NO at P<0.05.
b. significantly different from NS/NO at P<0.01.
c. significantly different from NS/NO at P<0.001.
analyzed. It may be concluded that opium consumption may have no, if any, adverse effects on sperm morphology. Some studies in animals [40] and a study on humans [41] have shown that chronic morphine administration have altered hypothalamic hormones and affected testicular function [42]. However, Cicero et al. suggested that the animals develop tolerance to the effects of morphine on reproductive hormones with long-term exposure [43]. Because the levels of Prolactin, LH, FSH, and testosterone were not evaluated as a routine paraclinical evaluation in subjects referred to the infertility clinic, it is not possible to suggest whether such a conclusion could be applied to human subjects. A precise study on opium-dependent subjects after evaluation of hypothalamic-pituitary-gonadal axis may elucidate the probable tolerance to chronic morphine in humans. However, the results of this study are in agreement with the study of Shuey et al. that found no adverse effects on rat sperm morphology and motility after 9 weeks treatment with different doses of oxymorphone (a potent opioid agonist) [44].

In this study, sperm progression was affected by opium consumption, in contrast to cigarette smoking that had no significant impact on sperm progression. By referring to Table II, it can be seen that subjects in NS/O group had a significant reduction in sperm progression. µ-opioid receptors have been detected in the head, the middle region, and the tail of sperm. In accordance with these results, incubation of spermatozoa with µ-receptor agonist morphine resulted in a significant decrease in sperm progression [45]. The authors suggest that sperm progression is affected by opium consumption, whereas heavy smoking increased [46] or did not change (our data) progression. A combined effect of opium consumption and cigarette smoking may be seen in S/O and O/RS groups in which the rate of progression was slightly but not significantly higher than the NS/O group.

Evaluating sperm concentration, motility, and viability, no significant difference was found according to the smoking habits or the opium dependence history, which is similar to the results that have been previously reported [11]. This is in contrast with other studies that suggested a negative impact of smoking on sperm motility and concentration [5, 34-37].

This study had some limitations: (i) the number of opium-dependent men who did not smoke cigarette was lower than the other groups, or the lack of smoking and opium consumption dose; (ii) alcohol consumption habit was not considered in this study because it is widely forbidden in Iranian society owing to religious issues and might not have biased the results. In a mini-review conducted on the impact of smoking and alcohol drinking on some sperm parameters, the concurrent use of alcohol and cigarette was reported in nine studies [24]. None of these studies had analyzed the joint effect of alcohol and smoking on reproductive system. However, experimental studies have shown a reduction in the number and motility of rat sperm following chronic exposure to nicotine and alcohol [47], and; (iii) although an attempt was made to exclude confounding factors from this study, some confounding factors such as accompanying diseases (mild varicocele, cancers, etc.) may be present that were not considered in this study. However, the large sample size of this study may have nullified the essence of such confounders. It is recommended that future

<table>
<thead>
<tr>
<th>Table 3. Results of semen analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS/NO</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Normozoospermia</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
</tr>
<tr>
<td>Oligozoospermia</td>
</tr>
<tr>
<td>Teratozoospermia</td>
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<tr>
<td>Asthenooligozoospermia</td>
</tr>
<tr>
<td>Asthenoteratozoospermia</td>
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<tr>
<td>Oligoteratozoospermia</td>
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<tr>
<td>Oligoasthenoteratozoospermia</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Values in parenthesis are percentages.
NS/NO, non-smoking and non-opium; S/NO, smoking and non-opium; NS/O, non-smoking and opium; S/O, smoking and opium and O/RS, opium-dependence regardless of smoking status.

* Significantly different from NS/NO at P=0.018.
studies may exclude as far as possible co-existing factors to nullify such confounding effects.

From this study, it can be concluded that cigarette smoking alters the semen quality and some sperm parameters as well. The impact of opium consumption on semen and sperm parameters was different from cigarette smoking.

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