THE EFFECT OF CHRONIC SMOKING ON SEMINAL PLASMA INSULIN-IIKE GROWTH FACTOR-I IN IDIOPATHIC MALE INFERTILITY

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INTRODUCTION
Spermatogenesis is a complex developmental process that depends on pituitary gonadotropins and testosterone. These hormones exert an indirect effect on spermatogenetic cells by locally produced autocrine, paracrine and juxtacrine regulations of testicular functions. They mediate interactions between Leydig cells, peritubular myoid cells, Sertoli cells and spermatogenetic cells as a pre requisite of maintenance and control of spermatogenesis. The physiological role of these factors in the testis and their regulatory function remain to be studied in detail (Glander et al., 1996).

One of the molecules that are believed to be involved in the development of germ cells are insulin like growth factor-I (IGF-I) (Spiteri Greech &Nieschlage, 1992; Loir &Le Gac, 1994), which is found in plasma and tissue fluids. Probably, the tissue IGF-I from paracrine / autocrine sources is more important than those from circulation (Lin, 1995).

IGF-I exerts a direct as well as an indirect influence on steriodogenesis (Lin et al., 1986; Saez et al., 1989), metabolism (Oonk et al., 1989), cell proliferation and differentiation (Borland et al., 1984; Soder et al., 1992) and is modified in its action by growth hormone (Lin, 1995). Testicular IGF-I is mainly produced in Sertoli cells (Ritzen, 1983) by stimulation of follicle stimulating hormone (Cailleau et al., 1990).

Because of the large number of
men worldwide who smoke and the fact that cigarette smoke contains known mutagens and carcinogens, Vine, (1996), indicated that cigarette smoking (using cotinine levels as the measure of tobacco smoke) is associated with modest reduction in semen quality including sperm concentration, motility and morphology. However, Gandini et al., (1997), suggested that nicotine and its metabolite cotinine are not responsible for the harmful effects of cigarette smoke on sperm kinetic parameters reported in other literature.

Landin-Wihelmsen et al., (1994), reported that blood-plasma IGF-I correlate negatively with the amount of tobacco smoked by men.

From the previous observations, this study was designed to evaluate the hazards of chronic cigarette smoking on semen quality and its relationship with seminal plasma IGF-I in idiopathic male infertility.

**SUBJECTS AND METHODS**

This study included (18) male subjects. They were selected from those patients attending Andrology Clinic, Mansoura University Hospital. The selected subjects are divided into two groups:

**Group I**: - included (9) Apparent healthy fertile non smokers with normal semenogram and proved fertility. They were selected as control group. The non-smokers are subjects who reported with minimal exposure to passive smoking.

**Group II**: - included (9) infertile smokers with abnormal semenogram for unknown etiology. The selected cases were isolated or combined oligo- asthenozoospermic (Count < 20 x 10⁶/ ml & motility <40% active forward progression) according to WHO (1993). They were selected as heavy smokers with deep puffs.

A thorough history was taken including age (aged 25-38 years), occupation, duration of smoking (more than 10 years), number of cigarettes per day (more than 20 cigarettes per day) as chronic heavy smoking according to Vine et al., (1996). Then, infertility scheme was fulfilled including duration of infertility (failed conception for at least one year with unprotected intercourse), sexual, developmental, medical, surgical and family histories, in addi-
tion to history of infections and gonadotoxins (Rowe et al., 1993). Full general and local examinations were done to exclude other possible causes of male infertility such as varicocele, hypogonadism, obstruction and cryptorchidism. Patients with disturbed serum hormonal profile and possible immunological factor were excluded. Scrotal ultrasound was done to exclude subclinical varicocele and other scrotal causes. Females from these couples exhibited normal ovulatory cycles with no abnormal changes in reproductive organs to exclude female factor as a possible etiology.

**Collection Of Semen Samples :-**

The samples were collected by masturbation into sterile plastic screw capped 50 ml. containers after sexual abstinence for 3-5 days. A single sample provided by each subject was examined by conventional methods (World Health Organization, 1993) and analyzed for volume, sperm count /ml., percentage of motile sperm, grading of motility and percentage of abnormal sperm forms (Rowe et al., 1993). All patients with samples showing evidence of genital tract infection were excluded from this study.

**Preparation of Seminal Plasma :-**

After liquefaction, cell free seminal plasma was obtained after an initial centrifugation (1000 g for 10 min.) followed by additional centrifugation of supernatant at 4000 g for 20 min. to remove the debris. Seminal Plasma aliquots were stored frozen at -20°C until the time of cotinine and IGF-I determination for each sample (Glander et al., 1996).

The seminal plasma samples were assessed for cotinine level by $^{125}$ I RIA assay (Langone et al., 1973) using kit provided from Diagnostic Products Corporation (DPC-USA). The results were expressed as nanograms of cotinine per milliliter of seminal plasma (ng / ml.).

The concentration of IGF-I was determined by a double antibody $^{125}$ I RIA method using the IGF-I immuno radiometric assay kit provided from Immunotech Coulter Company (France). The samples were extracted with acid ethanol to remove the IGF-I binding proteins (Kratzsch et al., 1993). The results were expressed as nanograms of IGF-I per milliliter of seminal plasma (ng / ml.).

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Statistical Analysis:-
Mean and standard error of mean (SEM) of variables were computed in each group, comparisons were performed by test for non-parametric data (Mann Whitney U-test). The level of significance was set at $P < 0.05$. Kendall correlation between seminal plasma cotinine, semen parameters and seminal plasma IGF-I was determined.

RESULTS
From data illustrated in table (1) & table (2), it was found that:

1- There was a significant decrease in sperm count / ml. ($P < 0.005$), percentage of motile sperm ($P < 0.0001$) and seminal plasma IGF-I levels ($P < 0.0001$), with significant increase in percentage of abnormal forms ($P < 0.005$) in smoked infertile subjects when compared with normal fertile group (table 1).

2- There was a negative correlation between seminal plasma cotinine levels and seminal plasma IGF-I levels ($P = 0.06$, nearly around the significant value), with non significant correlation with other semen parameters ($P > 0.05$) (table 2).
Table (1): Shows the effect of seminal cotinine level (secreted nicotine metabolite in semen) on volume of provided sample, sperm count/ml, percentage of abnormal forms, percentage of motile sperm and seminal IGF-I levels.

<table>
<thead>
<tr>
<th></th>
<th>Volume (ml)</th>
<th>Sperm count/ml (million/ml)</th>
<th>% of abnormal forms</th>
<th>% of motile sperm</th>
<th>Seminal IGF-I (ng/ml)</th>
<th>seminal cotinine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.39</td>
<td>89.44</td>
<td>18.67</td>
<td>75.33</td>
<td>128.89</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>± 0.34</td>
<td>± 7.15</td>
<td>± 0.75</td>
<td>± 1.96</td>
<td>± 12.37</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.5</td>
<td>90</td>
<td>19</td>
<td>75</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3-6</td>
<td>46-120</td>
<td>14-21</td>
<td>65-85</td>
<td>68-184</td>
<td></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.2</td>
<td>49.22</td>
<td>42.33</td>
<td>36.78</td>
<td>31.36</td>
<td>1110</td>
</tr>
<tr>
<td>SEM</td>
<td>± 0.54</td>
<td>± 10.3</td>
<td>± 4.95</td>
<td>± 6.41</td>
<td>± 4.92</td>
<td>± 498.62</td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>52</td>
<td>45</td>
<td>25</td>
<td>33</td>
<td>540</td>
</tr>
<tr>
<td>Range</td>
<td>2.8-7.5</td>
<td>2-88</td>
<td>18-60</td>
<td>20-66</td>
<td>8-58</td>
<td>230-5000</td>
</tr>
<tr>
<td>U</td>
<td>28.5</td>
<td>10</td>
<td>6</td>
<td>105</td>
<td>.000</td>
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</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

**Group I**: Control group

**Group II**: Infertile smokers

* Highly significant.

Mann-Whitney U-test.

Table (2): Shows Kendall correlation of seminal cotinine, semen parameters and seminal IGF-I (n=9).

<table>
<thead>
<tr>
<th></th>
<th>Volume</th>
<th>Count/ml</th>
<th>% of abnormal forms</th>
<th>% of motile sperm</th>
<th>Seminal IGF-I level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tb</td>
<td>P</td>
<td>tb</td>
<td>P</td>
<td>tb</td>
</tr>
<tr>
<td>Seminal cotinine</td>
<td>.343</td>
<td>.206</td>
<td>-.254</td>
<td>.35</td>
<td>.85</td>
</tr>
</tbody>
</table>

* Nearly around the significant value.
DISCUSSION

Table (1) illustrated that there was a significant decrease in sperm count / ml., percentage of motile sperm as well as significant increase in percentage of abnormal forms in infertile smoking subjects when compared with non smoking fertile group. These findings are previously reported by other investigators. However, the mechanism by which cigarette smoking might affect semen quality is not known.

Weisberg, (1985), reported that nicotine exposure in rats leads to atrophy of testis and impaired spermatogenesis. The fact that nicotine and cotinine, a metabolite of nicotine, are detectable in semen and responsible for impaired spermatogenesis was suggested by Pacifici et al., (1993) and Vine et al., (1994).

More recent studies assessed the relation between smoking and semen quality using semen cotinine levels as the measure of tobacco smoke and found that cigarette smoking is associated with lowered semen quality including total sperm count, percentage of motile sperm and increase percentage of abnormal forms (Vine, 1996; Zavos et al., 1998; Rubes et al., 1998 and Zenzes et al., 1999).

It was suggested that perhaps other compounds of tobacco smoke are diffusible to genital system and affect sperm development. For example, polonium-210, an α-emitting radioelement capable for damaging DNA and found in tobacco smoke, has been detected at higher concentrations in semen of smokers as compared with non smokers (Hunt, 1990). Exposure of male mice in utero to benzo(a) pyrene, a constituent of tobacco smoke, results in decrease testicular weight, atrophy of the seminiferous tubules and altered spermatogenesis (Mackenzie & Angevine, 1981).

Robbins et al., (1997), hypothesized that cytogenetic abnormalities in human sperm cells are associated with tobacco smoke. Also, Shen et al.,(1997), reported that cigarette smoking has deleterious effects on sperm quality more accurately than conventional seminal parameters. They found that the sperm of smokers contains levels of 8-hydroxydeoxyguanosine (80HdG), a major form of oxidative damage, in association with levels of seminal plasma cotinine which is a marker of smoking.
Although, Gandini et al., (1997), reported that nicotine and cotinine are not responsible for the harmful effects of cigarette smoke on sperm kinetic parameters reported in other literatures. However, Zenzes and Bielecki, (1998), suggested that the detection of nicotine and cotinine in semen of smokers constituents and/or DNA-reactive intermediates pass through the blood-testis barrier and react directly with germ cells leading to cytogenic abnormalities. Zavos et al.,(1998), illustrated that smoking a large quantity of cigarette per day (>20 per day) for prolonged period severely affected the axoneme of the human spermatozoon. These support our results as regards sperm motility disturbance.

Beside the studies that evaluate the correlation between cigarette smoke and semen quality, other studies in human indicate that cigarette smoke may alters levels of hormones that are involved in spermatogenesis as sex hormones and sex hormone-binding globulin in middle aged men (Field et al., 1994).

Sofikitis et al., (1995), studied the correlation between testosterone levels in blood serum and morphological sperm abnormalities. The secretory dysfunction of Leydig and Sertoli cells may be the cause of impaired sperm fertilizing capacity in smokers.

IGF-I is one of the hormones that are believed to play an important roles in male genital functions (Ritzen, 1983; Vanelli et al., 1988; Cailleau et al., 1990 and Vaughan & Vale, 1993).

The IGFs are mitogenic polypeptides that stimulate cellular proliferation and differentiation in addition to having metabolic effects (Langford & Miell, 1993). Sertoli cells, Leydig cells and peritubular cells secrete IGF-I (Cailleau et al., 1990). The cellular patterns of gene expression for these polypeptides suggested that they may play a significant roles in testicular functions and germ cell development (Zhou & Bondy, 1993). Glander et al.,(1996), found that IGF-I concentrations in seminal plasma showed a very close correlation with morphology of spermatozoa and sperm concentration, suggesting that IGF-I may function as a differentiation marker in male germ cells and has a role in maturation of spermatozoa. Also, Colombo & Naz, (1999), concluded that IGF-I levels in seminal plasma linearly correlated significantly with total.
sperm count. These finding suggested that IGF-I has a role in fertility and its derangement may be involved in male infertility, especially when mediated through low sperm count and immunological factors.

The significant changes in semen quality in infertile smokers illustrated in table (1), (significant decrease in sperm count / ml., significant decrease in percentage of motile sperm as well as significant increase in percentage of abnormal sperm) may be due to significant decrease in seminal plasma concentration of IGF-I.

These results are supported by Lee et al., (1995); Breier et al., (1996); Ovesen et al., (1996) and Ovesen et al., (1998). They found that seminal plasma IGF-I increased simultaneously with the increase in sperm motility. They suggested that growth hormone therapy stimulates IGF-I production from Sertoli and / or Leydig cells, which in a paracrine / autocrine manner stimulates maturation of spermatozoa with subsequent increased sperm motility.

Also, in our study the seminal plasma IGF-I concentration in infertile smokers was negatively correlated with seminal plasma cotinine. These finding suggested that reduced seminal plasma IGF-I may be a contributing factor in impaired semen quality and infertility in smokers.

The mechanism by which cigarette smoke decreases the seminal plasma levels of IGF-I is not studied yet and need further investigations.

From the results of this study and the previous observations of other workers, it is concluded that cigarette smoke has hazardous effects on male spermatogenesis either by direct effect on sperm kinetic parameters through the nicotine, its metabolites and other associated compounds or by decrease paracrine and / or autocrine IGF-I secretion in semen or by both mechanisms leading to impaired semen quality and infertility.

SUMMARY & CONCLUSION
This study was designed to evaluate the hazards of chronic cigarette smoking on semen quality and its relationship with seminal plasma IGF-I in infertile men.

This study included (18) male subjects. They selected from those patients attending Andrology Clinic.
Mansoura University Hospital. The selected subjects are divided into 2 groups. Group(1) included (9) male non smokers with normal seminogram and proved fertile. Group (2) included (9) infertile smokers for idiopathic cause.

After a thorough history and full clinical examinations were done, semen samples were collected from selected cases by masturbation after sexual abstinence for 3-4 days. The semen samples were examined by conventional method for volume, sperm count / ml., percentage of motile sperm and percentage of abnormal forms. After centrifugation of semen samples, the seminal plasma will be separated for assessment of cotinine and IGF-I by $^{125}$ RIA assay.

The results of this study showed that chronic smoking (using semen cotinine levels as the measure of tobacco smoke) led to significant decrease in sperm count / ml., percentage of motile sperm and seminal plasma levels of IGF-I with significant increase in percentage of abnormal sperm forms. Also, there is a negative correlation between seminal plasma cotinine levels and seminal plasma IGF-I levels, percentage of motile sperm and sperm count / ml. There is a positive correlation between seminal plasma cotinine levels and percentage of abnormal sperm forms.

According to the results of this study and the results of other investigators, it could be concluded that cigarette smoke has a hazardous effects on male spermatogenesis either by direct effect on sperm kinetic parameters through the nicotine, its metabolites and other associated compounds or by decrease paracrine and / or autocrine IGF-I secretion in semen or by both mechanisms leading to impaired semen quality and infertility.

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تأثير التدخين المزمن للسجائر على هرمون IGF-1

المريض: في الرجال المصابين بالعقم الغير مسبب

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د. أشرف حسن أحمد حسن

أ.د. صلاح محمد فايد

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كلية الطب - جامعة المنصورة

تم إجراء هذا البحث لدراسة تأثير التدخين الكثيف والمزمن للسجائر على كفاءة سائل المنى وعلاقته بهرمون IGF-1 في الرجال المصابين بالعقم.

يشمل هذا البحث 18 شخص من الذكور تم اختبارهم بناءً على الفحص الاكلينيكي. وقد تم تقسيمهم إلى مجموعتين، المجموعة الأولى وهي المجموعة الضارة بالدخان والمتوازنة من المرضى والمجموعة الثانية مجموعة ممتلكة وتوازنة من المرضى. وبعد ذلك تم رصد وتسجيل حالات فحص كل وتناسلي لاستيعاد الأسباب الأخرى المتوقعة للعقم وكذلك قياس هرمونات في السيرم والاختبارات المناعية في السائل المنى. تم أخذ عينات سائل المنى بعدة مرتين بعد أن تمت الاحتياطات اللازمة لذلك. وتم فحصها من حيث حجم العينة - عدد اليعانات المتنوعة لكل 1 ملليتر - نسبة اليعانات الحية - نسبة اليعانات المشوهة. ثم بعد ذلك تم عملية الفصل لاستخلاص بلازما سائل المنى حيث تم تجميع تركيز كل من الكورتيزون-17 ر في كل عينة.

وكانت نتائج البحث كالآتي:

- التدخين المزمن المصاحب بإفراز مادة الكورتيزون في السائل المنى أدى إلى انخفاض عدد اليعانات المتنوعة لكل 1 ملليتر ونسبة اليعانات المشوهة الحية وكذلك تركيز-17 ر في بلازما سائل المنى. وكانت النتائج ذات دلالة إحصائية واضحة. كما أنه أدى إلى زيادة نسبة اليعانات المشوهة أيضاً. وكانت النتائج ذات دلالة إحصائية واضحة.

- هناك علاقة سلبية بين تركيز الكورتيزون في بلازما سائل المنى وكل من تركيز-17 ر في بلازما سائل المنى، عدد اليعانات المتنوعة لكل 1 ملليتر ونسبة اليعانات المشوهة الحية.

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3- هناك علاقة إيجابية بين تركيز الكورتئين في بلازما سائل المنى ورنسية الحيوانات المشوهة.

ونستنتج من نتائج هذا البحث ومن نتائج الباحثين الآخرين أن تدخين السجائر له تأثير واضح على عملية تكوين الحيوانات المنوية سواء خلال تأثير النيكوتين أو مشتقاته المباشر على عملية التكوين أو من خلال عناصر أخرى مصاحبة للتدخين أو من خلال تأثيره على IGF-1 الذي يفرز موضعياً في الجهاز التناسلي للذكر.