Effect of Cigarette Smoke Exposure on Kisspeptin Levels in Pubertal Female Rats: Role of Vitamin D Supplementation

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Abstract

**Background:** Animal and human studies have shown associations between active smoking and altered female fertility. However, the reproductive effects of passive smoking is relatively new compared to the effects of active smoking. Pubertal exposure to cigarette smoke may be associated with reproductive effects in adulthood. There are some evidence that in addition to sex steroid hormones, the classic regulators of human reproduction; Vitamin D (Vit D) also modulates reproductive processes in women and men. Recently, the neuropeptide kisspeptin has been identified as a potential regulator of developmental changes in reproduction.

**Aim:** The present study was designed to evaluate the effect of cigarette smoke exposure on kisspeptin levels, Hypothalamic-Pituitary-Gonadal (HPG) axis in pubertal female rats. The effect of Vit D supplementation was also studied.

**Material and Methods:** Thirty female pubertal albino rats aged (35-40 days) were divided into three groups: Group (I) 10 rats served as control, Group (II) 10 rats exposed to cigarette smoke (1 hour/day) (CSE) and Group (III) 10 rats supplemented with Vit D in a dose of (650 IU/Rat/Day) along with Cigarette Smoke Exposure (CSE + Vit D). In adult female rats (85-90 days), serum levels of Vit D, kisspeptin, Gonadotropin Releasing Hormone (GnRH), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), estrogen and progesterone were detected. Ovarian levels of estrogen, progesterone, total antioxidant capacity and apoptotic marker (active caspase-3) were also determined.

**Results:** Cigarette smoke exposure caused a significant reduction in both serum levels of Vit D, Kisspeptin and hypothalamic-pituitary-ovarian hormones (GnRH, FSH, LH, estrogen and progesterone) and ovarian levels of estrogen, progesterone and total antioxidant capacity. Significant elevation in active caspase-3 was observed. On the contrary, Vit D supplementation caused a significant elevation in serum levels of kisspeptin, hypothalamic-pituitary-ovarian hormones and ovarian levels of estrogen, progesterone and total antioxidant capacity. However, significant reduction in apoptotic marker (caspase-3) was detected. Kisspeptin was positively correlated with Vit D in (CSE) and (CSE + Vit D) groups and with ovarian estrogen in (CSE) group $p<0.001$. On the other hand its inversely correlated with active caspase-3 in both (CSE) and (CSE + Vit D) groups.

**Conclusion:** According to the findings of the present study, it can be concluded that exposure to cigarette smoke during pubertal period may lead to reduction in Vit D. This could impair kisspeptin level, inducing hypothalamic dysfunction, and affect pituitary and ovarian function. On the other hand, vitamin D supplementation modulates kisspeptin function which preserves hypothalamic-pituitary-gonadal axis, prevents oxidative damage, loss of ovarian function and reduces ovarian apoptosis. Therefore, we could suggest that Vit D may have an important protective role against deleterious effects of cigarette smoke exposure. It could be considered as a key regulator of neuroendocrine and ovarian function.

**Key Words:** Cigarette smoke exposure – Kisspeptin – Hypothalamic-pituitary-gonadal axis – Vitamin D supplementation.

Introduction

CIGARETTE smoking is the most preventable cause of morbidity and premature mortality worldwide. Smoking introduces a wide range of diseases including, but not limited to, many types of cancer, cardiovascular diseases and respiratory diseases [1]. Although, animal and human studies have shown associations between active smoking and altered female fertility and embryo development [2], the reproductive effects of secondhand smoke exposure is relatively new compared to the effects of active smoking.

Secondhand Tobacco Smoke (STS), passive smoking also known as environmental tobacco smoke is a mixture of over 4000 chemicals, more than 60 of which are known or suspected carcinogens or reproductive toxicants (e.g. carbon monoxide, cadmium, lead, benzene, nicotine, radioactive polonium-210 and Polycyclic Aromatic Hydrocarbons (PAHs) which were found to be higher in Sidestream Smoke (SS) than mainstream smoke (MS) (about 10-fold) [3]. Some studies have indicated significantly higher levels of smoking toxicants in reproductive tissues or fluids than in serum, which suggested that the toxicants accumulated in the reproductive organs [4].
Non-smoking women who are exposed to second-hand smoke are at increased risk of difficulty becoming pregnant, of giving birth prematurely of stillbirth [5], of spontaneous abortion [6], and of having a baby with congenital malformations [3]. Moreover, female fertility can be damaged in utero if the woman’s mother was exposed to secondhand smoke while pregnant [7]. There is also a new evidence that developmental exposures to tobacco smoke may be associated with reproductive effects in adulthood [8]. However, the complex processes by which cigarette smoke exposure may deleteriously affect physiological mechanisms underlying sexual function is not clearly understood.

At puberty, fertility is initiated by the pulsatile secretion of gonadotropin releasing hormone (GnRH) from a small number of neurons in the hypothalamus. Although GnRH neurons are a critical component of the reproductive axis, Kisspeptin (Kp) peptides have been identified recently as vital upstream regulators that integrate central and peripheral signals with GnRH release, thereby playing a pivotal role in the control of reproduction [9].

Kisspeptin, a hypothalamic peptide, coded by the Kiss 1 gene, is a novel neuromodulator sensitive to sex steroid feedback and metabolic cues. Kisspeptin is now recognized as a crucial regulator of the onset of puberty. Kisspeptin expression has been identified in multiple tissues, including pancreas, adipose tissue, gonads, and placenta, however, its main functional role is mediated by its expression within the central nervous system [10].

During the last decades, the outlook on vitamin D has widened, from being a vitamin solely involved in bone metabolism and calcium homeostasis, to being a multifunctional hormone known to affect a broad range of physiological processes [11]. There is some evidence that in addition to sex steroid hormones, the classic regulators of human reproduction, Vit D also modulates reproductive processes in women and men. It has been reported that Vitamin D Receptor (VDR) knockout mice have significant gonadal insufficiency, decreased sperm count and motility, and histological abnormalities of testis, ovary and uterus. Experimental studies have demonstrated that ovaries is a target organ for Vit D raising the possibility that vit D might play a role in modulating ovarian activity [12]. However, the mechanisms by which vitamin D regulates female reproduction is minimally understood.

Therefore, the present study was designed to evaluate the effect of cigarette smoke exposure on kisspeptin levels, Hypothalamic-Pituitary-Gonadal (HPG) axis in pubertal female rats. The effect of Vit D supplementation was also studied.

Material and Methods

Ethics statement:

This study was approved by the Ethics committee of the Medical Research Institute, Alexandria University.

Experimental animals:

Thirty new born female albino rats were obtained from the animal house of Medical Research Institute, Alexandria University, Egypt. Rats were maintained in polycarbonate cages at 22 ±2°C on a 12-hour light-dark cycle and were provided with food, and tap water ad libitum throughout the experiment.

Visual inspection of the vulva was performed until detection of vaginal opening, the external sign of puberty. Pubertal animals (35-40 days of age) were divided into three groups: Group (I) 10 rats served as control, Group (II) 10 rats exposed to cigarette smoke for 1 hour/day (passive smoking) [13], Group (III) 10 rats exposed to the same time of cigarette smoke along with Vit D3 supplementation in dose of (650IU/rat/day).

The adult female rats (85-90 day of age) were killed by decapitation, then trunk blood and ovaries were obtained from all studied groups. Blood samples were collected and centrifuged at 1500rpm for 15 minutes. Sera were aliquoted and Vit D levels were determined immediately. Remainder sera were stored at −80°C.

Cigarette smoke exposure:

Rats were placed in a glass smoking chamber (closed box of 20cm length, 40cm breadth and 25cm width). This box was airtight, having holes in two opposite sides and was covered by a glass lid. A cigarette was fixed into a hole where the lit end was introduced into the box and the other end fixed in a pump. Thus, by suction the cigarette smoke is introduced into the box. The type of cigarette used was a filter tipped containing 0.8mg of nicotine/cigarette.

Preparation of ovarian homogenate:

Ovaries were dissected, cleaned from adhering matter, their weights were recorded and then homogenized in (0.015 M Na2HPO4, 0.15 M NaCl) buffer at PH 7.8 (10% homogenate W/V) using Teflon automatic homogenizer. The supernatant obtained from the homogenate by ultracentrifugation and filtration was kept at −80°C until biochemical determination.
Biochemical assays:

Serum level of 25-OH Vitamin D was determined by using ELISA Kit. Serum level of Gonadotropin Releasing Hormone (GnRH) was determined using a double antibody sandwich type of Enzyme-linked Immunosorbent Assay (ELISA) using kits purchased from Kamaiya Biomedical Company. Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were determined using Electro-Chemiluminescence Immunoassay (ECLIA) employing two different FSH and LH specific monoclonal antibodies and kits purchased from Roche Diagnostic. Serum kisspeptin was determined by using ELISA kit. Estrogen and progesterone levels were determined in both serum and ovarian homogenate by using (ECLIA) assay. Colorimetric method was used to determine ovarian total antioxidant capacity [14]. Active caspase-3 was determined in ovarian homogenate by using ELISA kit.

Statistical analysis:

Data were analyzed using Statistical Package for Social Sciences (SPSS). Normally distributed quantitative data were expressed in mean ± SD and were compared using F test (ANOVA) and Post hoc Least Significant Difference test (LSD) for pairwise comparison, while abnormally distributed data were expressed in median, (Min.-Max.) and were compared using Kruskal Wallis test and Post Hoc test (LSD) for pairwise comparison, while abnormally distributed data was expressed in median, (Min-Max) and was compared using Kruskal Wallis test and post Hoc Least Significant Difference test (LSD) for pairwise comparison. For all statistical tests, level of 5% was considered significant.

Results

Table (1) shows body weights, serum levels of Vit D, kisspeptin, GnTR, FSH, LH, estrogen and progesterone. In addition to ovarian levels of estrogen, progesterone, total antioxidant capacities and active caspase-3 in the different studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Group I) (n=10)</th>
<th>(CSE) (Group II) (n=10)</th>
<th>(CSE+Vit D) (Group III) (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Vit D (ng/ml)</td>
<td>29.10±6.19</td>
<td>23.77±2.97</td>
<td>35.95±6.48</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum kisspeptin (pg/ml)</td>
<td>178.20 (110.4-210.5)</td>
<td>186.16±123.3-161.2</td>
<td>218.3±204.4-230.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GRH (pg/ml)</td>
<td>19.4±2.93</td>
<td>9.02±2.48</td>
<td>18.4±1.62</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FSH (ng/ml)</td>
<td>15.1±3.25</td>
<td>6.13±2.0</td>
<td>14.4±1.23</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>5.62±1.16</td>
<td>1.95±0.91</td>
<td>6.48±1.39</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum estrogen (ng/ml)</td>
<td>40.5 (35.0-60.0)</td>
<td>27.0±(12.0-31.0)</td>
<td>52.3±(47.0-65.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum progesterone (ng/mL)</td>
<td>7.74±1.01</td>
<td>3.44±1.12</td>
<td>7.87±1.15</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ovarian estrogen (ng/g tissue)</td>
<td>71.50 (69.0-75.0)</td>
<td>50.0 (33.0-52.0)</td>
<td>104.0 (90.0-155.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ovarian progesterone (ng/g tissue)</td>
<td>18.6±1.12</td>
<td>5.86±3.0</td>
<td>27.3±6±2.03</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>T. antioxidant capacity (mM/g tissue)</td>
<td>39.80 (34.2-41.0)</td>
<td>34.0 (29.0-36.0)</td>
<td>61.5 (59.0-64.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ovarian caspase-3 (ng/g tissue)</td>
<td>1.65±0.29</td>
<td>2.42±0.34</td>
<td>1.55±0.25</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Significant reduction in the mean values of body weight of rats exposed to cigarette smoke (CSE) than control and Vit D supplemented groups (CSE+Vit D) was detected (p<0.001).

Exposure to cigarette smoke cause a significant reduction in serum level of Vit D from 29.1 ±6.19 to 23.77±2.97 in control group, however, oral supplementation with Vit D elevated this level to 35.95±4.68 (p<0.001) Fig. (1). Serum levels of kisspeptin in the different studied groups are represented in Fig. (2). Significant reduction in kisspeptin level was detected in (CSE) group. However, its level was significantly elevated in (CSE+Vit D) group than both control and (CSE) groups. Serum levels of GnRH, FSH, LH, estrogen and progesterone were significantly lower in (CSE) group as compared to control (p<0.001).

Ovarian estrogen, progesterone and total antioxidant capacity were reduced in (CSE) group as compared to control (p<0.001). However, significant elevation in active caspase-3 was detected in the same group Figs. (3-6).

Oral supplementation with Vit D3 to cigarette smoke exposed female rats cause an elevation in serum levels of FSH, LH, estrogen and progesterone (hypothalamic-pituitary-ovarian hormones) p<0.001.

In addition, significant elevation in ovarian estrogen, progesterone and total antioxidant capacity were detected. However, caspase-3 was significantly reduced in the same group as compared to control (p<0.001). Therefore, Vit D may have a protective role against deleterious effects of cigarette smoke exposure.

Ovarian weights:

Although, there were a slightly decrease in ovarian weights in (CSE) group as compared to control and (CSE+Vit D) this reduction was not statistically significant.

Table (1): Serum and ovarian biochemical parameters in the different studied groups.

Normally distributed quantitative data was expressed in mean ± SD and was compared using F test (ANOVA) and Post hoc test (LSD) for pairwise comparison, while abnormally distributed data was expressed in median (Min.-Max.) and was compared using Kruskal Wallis test and was assessed using Mann-Whitney Test. a: Significant with Group I. b: Significant with Group II. *: Statistically significant at p≤0.001.
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**Correlation studies:**

Table (2) shows correlations between serum kisspeptin levels and Vit D, ovarian estrogen, progesterone, total antioxidant capacities and caspase-3 in the different studied groups. Significant positive correlations between kisspeptin and Vit D in both (CSE) and (CSE+Vit D) were detected \((p=0.001, <0.001)\) respectively. Serum kisspeptin levels were positively correlated with ovarian estrogen in (CSE) group \((r=0.863, p=0.001)\) and with total antioxidant capacities in both (CSE) and (CSE+Vit D) \((r=0.749, p=0.013, r=0.963, p<0.001)\) respectively. There were negative correlations between kisspeptin and ovarian caspase-3 in (CSE) and (CSE+Vit D) \((r=0.988, p<0.001, r=0.902, p<0.001)\) respectively.
Table (2): Correlations between serum kisspeptin levels, Vit D and different ovarian parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control Group I</th>
<th>(CSE) Group II</th>
<th>(CSE+Vit D) Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum kisspeptin (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit D (ng/ml)</td>
<td>0.012</td>
<td>0.973</td>
<td>0.837*</td>
</tr>
<tr>
<td>Estrogen (ng/g tissue)</td>
<td>-0.373</td>
<td>0.288</td>
<td>0.862*</td>
</tr>
<tr>
<td>Progesterone (ng/g tissue)</td>
<td>-0.616</td>
<td>0.058</td>
<td>0.061</td>
</tr>
<tr>
<td>Total antioxidant capacities (mM/g tissue)</td>
<td>-0.098</td>
<td>0.789</td>
<td>0.749*</td>
</tr>
<tr>
<td>Caspase-3 (ng/mg protein)</td>
<td>0.141</td>
<td>0.697</td>
<td>-0.988*</td>
</tr>
</tbody>
</table>

$r_s$: Spearman coefficient. *: Statistically significant at $p \leq 0.05$.

Discussion

It has been reported that cigarette smoke harms the reproductive system in many aspects. It impairs every stage of the reproductive process and each part of the reproductive system such as folliculogenesis, steroidogenesis, embryonic development and transport. Cigarette smoke compounds interact with different reproductive targets, depending on individual sensitivities, the presence of other toxic substances and according to time, dose, type and duration of exposure [15].

In the present study, exposure to cigarette smoke during pubertal period cause a reduction in serum levels of Vit D and kisspeptine, alteration in hypothalamic-pituitary-gonadal axis, disturbance in ovarian hormones and elevation in ovarian oxidative stress and apoptosis.

In accordance with the results of the present study, Vidal et al., [16] suggested that cigarette smoke is a reproductive toxicant that may cause deleterious effects on ovarian function and sex steroid hormone levels. Smoke compounds disrupt steroidogenesis, leading to impairment of estrogen (E2) synthesis and progesterone synthesis deficiency. Moreover, in a smoking model, lower levels of Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Gonadotropin Releasing Hormone (GnRH) were noticed [17].

It is well known that cigarette smoking can harm fertility, but the existing research has targeted primarily on ovarian follicles, embryos or sex hormones [18]. Further studies investigating the mechanisms underlying cigarette smoking and fertility concerned the effects of the inherent toxicant molecules on follicles: For example, Benzo[a]pyrene (BaP), a component of cigarette smoke; caused few of ovarian follicles [1], rupture in the granulose cells, mitochondrial leakage [19], reduced oolemma fluidity, impaired fertilization in adulthood and aging of oocyte and dysfunction. Matsumawa et al., [20] added that activation of Aryl Hydrocarbon Receptor (AhR) by BaP stimulates vitamin D3 catabolism. Polycyclic Aromatic Hydrocarbons (PAHs) reduced numbers of primordial and primary follicles in rats and mice. Moreover, exposure to toxicological levels of (PAHs), results in follicle loss by apoptosis [21].

Cadmium (Cd), a heavy metal compound in cigarette smoke, is an endocrine disruptor which is involved in the impairment of steroidogenesis, it has a long biological half-life and accumulates over time in ovaries. In porcine granulosa cell lines exposed to low doses of Cd, increased genomic expression of P450 Side-Chain Cleavage (scc) enzyme, involved in the conversion of cholesterol to pregnenolone. At high doses, Cd was shown to inhibit P450scc expression, resulting in decreased E2 synthesis. Moreover, high doses of Cd led to granulosa cell necrosis [2,22]. In addition to nicotine damage on granulose cells, which has been reported as promoting cell apoptosis [15].

In accordance with the result of the present study, Durana et al., [23] reported that there is an increase in ovarian apoptosis with smoking. On the contrary, Tuttle et al., [24] mentioned that exposure to cigarette smoke does not increase the rates of apoptosis in the ovary, and by suggesting that there is an increased rate of follicle recruitment.

Cigarette smoke may have detrimental effects on oocyte through inducing oxidative stress and injuring granulosa cells. This was explained by Siddique et al., [25] who reported that a toxic follicle environment increased oxidative stress with decrease in SOD activities, inducing abnormal intercellular cross-talk, meiosis impairment and activa-
tion of cell death pathways. Oxidative stress reflects serious consequences, for instance, enzymatic inactivation, DNA fragmentation, and irreversible damage of mitochondrial DNA, membrane lipids, and proteins resulting in mitochondrial dysfunction and ultimately cell death.

In chronically Vit D deficient females, increased expression of genes responsible for enzymes involved in cellular antioxidant defense systems suggest higher levels of intra-ovarian oxidative stress. A decrease in Gsr expression, which is a sulfur-redox cycle enzyme important in maintaining a balanced redox state and reduced free radical formation, could also contribute to the poor fertility outcomes seen in Vit D deficient populations [26]. Other studies mentioned that the effects of cigarette smoke on granulosa cells and aromatase enzyme decreases estrogen production, and the alkaloids in cigarette inhibit the production of progesterone [27].

It has been established that Vit D acts as a regulator of a number of enzymes involved in the regulation of steroid hormones production, and thereby the production of both adrenal steroid hormones and sex hormones [28]. Vit D alters the aromatase activity in placental cells [29] and prostate cells [30]. Vitamin D receptor null mutant mice have a decreased aromatase activity in the ovary, testis and epididymis. In breast cancer cell lines, Vit D treatment resulted in decreased aromatase gene expression, while the same treatment increased the aromatase gene expression in osteosarcoma cell lines. Therefore, vitamin D3 has been proposed to be a tissue-selective aromatase modulator [11].

Vitamin D deficiency may exacerbate symptoms of Polycystic Ovarian Syndrome (PCOS), associated with ovulatory and menstrual irregularities and lower pregnancy success. This is not corrected by normalizing the hypocalcemia in Vit D-deficient female rats, but requires vit D [32]. In the present study Vit D supplementation along with cigarette smoke exposure reduced ovarian apoptosis, protects ovarian tissues from oxidative stress, increases kisspeptin and then, regulates the reproductive axis. Kisspeptin was positively correlated with vit D and negatively correlated with apoptotic marker (active caspase-3) in both Cigarette Smoke Exposed (CSE) and Vit D supplemented exposed (CSE+Vit D) groups. It has been established that, in normal tissues vitamin D plays an important role in promoting apoptosis. It regulates apoptosis according to the requirements of the body at different physiological stages [12].

Moreover, the expression of VDR mRNA in the ovaries, in mixed ovarian cells, and in purified granulosa cell cultures indicating a role of Vit D in steroidogenesis. In human ovarian tissue, Vit D stimulated progesterone production by 13%, estradiol production by 9% and estrone production by 21% [11].

It has been established that sex steroids play a major role in regulation of serum kisspeptin level which in turn affects fertility. The majority of kisspeptin neurons express estrogen, progesterone and androgen receptors, consistent with their role as mediators of sex steroid feedback on reproductive axis [32].

In the present study a positive correlation between ovarian estrogen and serum kisspeptin was detected in cigarette smoke exposed group only; however, no correlation was detected in Vit D supplemented exposed group. This may be due to the direct effect of Vit D on kisspeptin level. Therefore, we can suggest that Vit D has direct and indirect (by enhancing estrogen level) effects on kisspeptin level.

A recent study demonstrated that E2 is essential for the prepubertal development of kisspeptin peptide and suggested that an E2-kisspeptin positive feedback mechanism exists before puberty. This implies kisspeptin neurons are E2-dependent amplifier of GnRH neuron activity in the prepubertal period. Estradiol is responsible for initiating kisspeptin expression in periventricular hypothalamic neurons that are thought to activate gonadotropin-releasing hormone neurons controlling puberty onset [33].

In support of this hypothesis, some investigators demonstrated that kisspeptin 1 neurons are virtually absent in estradiol-deficient, aromatase knockout mice. Thus, it is possible that the pubertal increase in Kiss1/Kiss1r mRNA in females is due to the increase in estrogens at this stage of development [34]. In addition, due to the regulatory role of kisspeptin on GnRH neurons, it is thought that kisspeptin may play a major role in pulse generation. In rats, administration of a kisspeptin antagonist directly into the arcuate nucleus caused a profound reduction in the number of LH pulses generated [35]. Kisspeptin is a principal regulator of the secretion of gonadotropins, and through this key role it is critical for the onset of puberty, the regulation of sex steroid-mediated feedback and the control of adult fertility [36]. Kisspeptin cells in the Arcuate nucleus (Arc) appear to receive and forward signals applicable to negative feedback regulation of
GnRH. In the female rodent anteroventral periventricular nucleus (AVPV) kisspeptin cells are important for positive and negative feedback regulation of GnRH and their number in female mice progressively increases until the age of puberty [37,38]. Additionally, it appears that in female rodents an estrogen-dependent developmental increase in kisspeptin peptide and mRNA in the AVPV occurs, leading presumably to an increase in the secretory activity of this rostral population of kisspeptin neurons. Physiological and pharmacological studies indicating that kisspeptin is the most potent GnRH secretagogue [32].

It was mentioned that Vit D regulates human chorionic gonadotropin expression and secretion in human syncytiotrophoblasts and increases placental sex steroid production [39]. In humans, GnRH and its receptor have been found in extra-pituitary tissues such as the ovary, breast and placenta. In the ovary, the expression of GnRH receptors has also been demonstrated in human ovarian granulosa cells [40]. Moreover, kisspeptin has a role in reproductive neuroendocrine signaling outside of the brain which suggested by the finding of both Kiss 1 and Kiss1r in pituitary gonadotrophs. Thus kisspeptin may regulate reproductive function at both hypothalamus and pituitary level [41].

Other study demonstrated that GnRH neurons express Vitamin D Receptor (VDR) protein, raising the possibility that Vit D3 directly regulates GnRH neurons. Moreover, VDR are found in the gonads, hypothalamus, and pituitary, suggesting that the reproductive axis may be regulated by paracrine and/or autocrine activities of Vit D [42].

Therefore, we propose that Vit D deficiency most likely impairs female reproductive function by inducing hypothalamic dysfunction, which secondarily affects pituitary and ovarian function. It was reported that, in the nervous system, the active Vit D affects the conduction of the motor neurons and synthesis of neurotrophic factors, thus preventing damages to the neurons. In addition, Vit D reduces markers of oxidative stress in vascular and nervous tissues. Additionally, developmental Vit D deficiency is hypothesized to adversely affect neurodevelopment [43,44]. These data suggest that vitamin D is a key regulator of neuroendocrine and ovarian function.

Finally, findings of the present study indicate that cigarette smoke is a high risk for female reproductive system. Exposure to cigarette smoke may cause a reduction in Vit D. This could impair kisspeptin level, inducing hypothalamic dysfunction, and affect pituitary and ovarian function. On the other hand, Vit D supplementation modulates kisspeptin function which preserves the hypothalamic-pituitary-gonadal axis. In addition, Vit D reduces ovarian apoptosis, prevents oxidative damage and loss of ovarian function. This occurs through its direct and indirect roles on kisspeptin.

Therefore, we could suggest that Vit D may have an important protective role against deleterious effects of cigarette smoke exposure. We certainly recommend that quitting exposure to cigarette smoke is a wise choice to ensure good fertility. The determination of optimal Vit D levels in the pubertal period and the amount of vitamin D supplementation required to achieve those levels for the numerous actions of vitamin D throughout a female’s life would have important public health implications.

References


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الملخص العربي

أوضحت الدراسات الحيوانية والدقيقة وجود علاقات بين التغذية الإيجابية والخلل في الخصوبة النسائية. بالرغم من أن تأثير التدخين السلبي على الكثيرون يعتبر جديد نسبياً بالمقارنة بالتدخين الإيجابي، إلا أنه يمكن أن يكون التعرض لدخان السجائر في مرحلة البلوغ الأولى له تأثيرات في المرحلة الناضجة للبلوغ، وتشير بعض الأدوات أن él بالإضافة إلى الهرمونات الجنسية المنظمة الأساسية للتكاثر فإن فيتامين D له دور محوري لعلاج تأثير التدخين والرها.

الهدف: وضعت هذه الدراسة لقيم تأثير التعرض لدخان السجائر على مستوي كل من الكسيبيتين والمحور الهيپوتكايسي-النخامي التانسالي في فيتامين D في الدم، وذلك بدراسة تأثير التدخين على الأداء في سيتيديم.

المؤلفون: الخطاب التأسيسي لموروث البيكسيتيما في فتامين D، ودور التدخين في الدم، ومن ثم تأثير التدخين على الأداء في سيتيديم.

المستقبل: مراجعة حديثة للنتائج المكملة في düzائين VITAMIN D، ودور التدخين في الدم، ومن ثم تأثير التدخين على الأداء في سيتيديم.

المستقبل: مراجعة حديثة للنتائج المكملة في düzائين VITAMIN D، ودور التدخين في الدم، ومن ثم تأثير التدخين على الأداء في سيتيديم.