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RESEARCH ARTICLE

Effect of passive smoking on heavy metals concentration in blood and follicular fluid of patients on going ICSI

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Abstract

Purpose: To assess the effects passive smoking and heavy metal concentrations in the blood and follicular fluid on assisted reproductive technology outcome.**Methods:** A prospective study was conducted between March 2017 and January 2018 in population consisted of 75 female patients undergoing an In vitro fertilization (IVF) cycle at Dr. Faris Medical center for Infertility and Human Reproduction located on Heliopolis, Cairo, Egypt; with unexplained infertility who underwent intracytoplasmic sperm injection (ICSI) using GnRH-antagonist protocol. Concentrations of three toxic metals Cadmium (Cd), Lead (Pb) and Arsenic (As) were measured both in blood sera and follicular fluid specimens. Patients were evaluated in two groups both undergoing ICSI; the first group consisted of patients who does not smoke or exposed to smoking (n=28) and the group passive smoker females (n=47). **Results**: These three heavy metals were significantly higher in serum and follicular fluid of passive smoking females (P<0.05). Also, higher concentrations of Cd, Pb and As were found in follicular fluid of passive smoking patients in comparison with non-smoker females. However, concentrations of Cd and Pb were not significantly different between both serum and follicular fluid. **Conclusion**: Passive smoking could affect levels of Cd, Pb and As in serum and follicular fluid of females undergoing Intra Cytoplasmic Sperm Injection (ICSI).

Keywords: Cadmium (Cd); Lead (Pb); Arsenic (As); Follicular fluid (FF); Intra Cytoplasmic Sperm Injection (ICSI); heavy metals

1 Introduction

Smoking-related diseases can be attributed to the inhalation of a variety of toxins in cigarette smoke, including nitrosamines, polycyclic aromatic hydrocarbons, volatile organic compounds, and several heavy metals[1]. Arsenic (As), Cadmium (Cd), Lead (Pb) and are among the most common heavy metals associated with the adverse health effects of smoking[2]and are all designated as carcinogenic to humans by the International Agency for Research on Cancer.[3] Heavy metals are metallic elements that have a relatively high density compared to water and could adversely affect human beings and animals as well as vegetation .[4] Although some heavy metals are necessary for life, most are considered non-essential and some have adverse health effects to humans.[5] Accumulation of heavy metals in the reproductive tissues of both women and men corroborates the likelihood of reproductive toxicity of heavy metals.[6] Likewise, an association had been reported between heavy metals (like As, Cd and Pb) and increased oxidative stress, cell apoptosis, endocrine disruption and epigenetic damage and through these mechanisms of toxicity, such elements could adversely affect outcomes of patients undergoing ICSI.[7]

Follicular fluid is comprised of a blood plasma ultra-filtrate, with selective exclusion of high weight molecular proteins facilitated by the blood-follicle-barrier that fills the growing follicle and bathes a developing oocyte[8]. The constituents of follicular fluid may reflect environmental exposures relevant to the early stages of human reproduction including the quality of a developing oocyte and embryo and it is presumed that follicular fluid provides a closer approximation to the biologically effective dose of a toxicant to an oocyte than achieved using blood or urine measures[9]. Moreover, Kruger et al.[10] clarified that the follicular fluid surrounds the oocyte (the developing egg, in the ovarian follicle,) it is composed of high concentrations of proteoglycans and steroids, and since it increases in volume as the follicle matures, high metabolic activity is characteristic of the matrix. So, controlling exposure to such toxic elements could be of interest to treat couples with reduced fertility, and increase the likelihood of success in assisted reproduction techniques[7]. The present study was to clarify the effect of passive smoking on the of heavy metals concentration in blood and follicular fluid of female patient ongoing ICSI. Also, we explored differences between concentrations of Cd, Pb and As in serum and follicular fluid of females in non-smoker group and passive smoker group undergoing ICSI.

2 Material and methods

Patient selection

Cases with known infertility causes should be treated before IVF, whether the cause was from female side or male side. Thus these cases were excluded from our study. Therefore, the cases chosen in our study are all of unknown cause of infertility. Heavy metals might be a potential cause of infertility. This is the rationale of patient selection. The study population consisted of 75 female patients undergoing an IVF cycle at Dr. Faris Medical center for Infertility and Human Reproduction located on Heliopolis, Cairo, Egypt, throughout a period of 3 years (36 months). Case history was recorded for all patients and females whose husband is smoker were considered as passive smoking females. Patients were classified according their history (non-smoking or smoking husbands) into two groups as follows: non-smoking females (n = 28), passive smoking females (n = 47). This is why the different size of both groups. The total period of exposure for the passive smoking females extends from 1 to 4 years with an average exposure of 2.38$\pm$ 0.157 years. All passive smoking females were treated as one group regardless the exact period of exposure. A total of 75 cycles of 75 couples enrolled ICSI program in the Centre Inclusion Criteria: (i) infertile women with an indication for ICSI including unexplained infertility, tubal factor (including treated hydrosalpinx and pyosalpinx), and male factor (including concentrations down to 5 million – 10 million sperm/ml); (ii) the couples were exposed to pollution by smoking or other passive smoking depends of their life style; (iii) the woman being 40 years or younger; (iv) at least 10 months of marriage without normal pregnancy; (v) women with Body Mass Index (BMI) 18-29.

Consent

Eligible women were counseled and offered to join the study after obtaining a written informed consent. The consent forms were signed after thorough discussion with the couples and explanation of the study purpose.

Serum collection

Venous blood samples were obtained following the standard procedure, using tubes with a clotting activator (S-Monovette, Sarstedt, No: 04.1905) for serum preparation. Serum samples were centrifuged at 2000xg for 15 min to separate the serum from the plasma. Specimens were immediately frozen (-80°C) until further use for assessment of levels of different heavy metals in the lab of Regional Center for Fungi and its Application- Faculty of Science, Al Azhar university.

Follicular fluid retrieval

The patient reports to the assisted conception unit on the morning of the procedure. She should be fasting for at least five hours; she is usually advised not to have any food or drink from the preceding midnight. She is prepared in the ward and taken to the procedure room of the unit. V9arious forms of anesthesia can be used but short-acting intravenous sedatives and narcotics are popular such as propofol combined with alfentanil. The vagina can be cleansed with antiseptics and irrigated with normal saline to remove traces of the antiseptic. There is however no guarantee that the irrigation will remove all traces of the antiseptic which if it comes into contact with the oocytes may exert toxic effects. Alternatively, the vagina can be wiped of all mucus with gauze swabs soaked in normal saline and no increase in pelvic infection has been noted with this cleaning method especially if this is performed in conjunction with antibiotic prophylaxis. A transvaginal ultrasounds is carried out and oocytes were aspirated from follicles in both ovaries through a needle that is used to pierce the vaginal wall and puncture the follicle. Each tube of aspirated fluid is examined under stereo magnification (Leica M80 Germany) to identify the oocyte which was then removed and washed in clean culture medium. The retrieved oocytes were placed in culture dishes.

Follicular fluid preparation and freezing

The follicular fluid was obtained from the largest follicle (> 18 mm) visualized on ultrasound before using any flushing medium and only consisted of fluid from one follicle. This follicle was aspirated using a transvaginal ultrasound probe with a 17 gauge, 35 cm oocyte aspiration needle (Cook® Double Lumen Aspiration Needle, USA) mounted on a needle guide directly attached to the probe. Between 0.5 mL and 5.0 mL FF was aspirated from a single large follicle in each ovary. The follicular fluid was transferred to a sterile Petri dish, and after the oocyte removal, the fluid was placed into a 15 mL conical tube and centrifuged for 5 minutes at 1500 g, distributed into 1.8 mL polypropylene cryo-vials, and stored at 80°C until further use[11].

Trace elements determination

The analysis of human follicular fluid for trace elements was approved by the Fungi Research Center, Faculty of Science, Al Azhar University. Samples had been submitted for analyzing the concentration of Cd, Pb and As by Atomic Absorption (Flame Method). The data were expressed in mg/L (ppm) using Atomic Absorption Spectrophotometer (model: GBC932AA).

Digestion of samples

Milli-Q water (ultra-pure water) was used to prepare all solutions. All other reagents were purchased from Sigma–Aldrich. The trace elements were determined after digestion with nitric acid 0.5%.

1. 1mL of sample added to 4mL of Mix. Acid (nitric + perchloric) 1:1
2. Heat mix with sample until dryness (about 15 Min)
3. Add 2 mL Milli-Q (0.5% nitric).

Statistical analysis

All statistical analyses were performed using the statistical package for social science (SPSS) 24.0 for windows. Statistical analysis for effect of passive smoking on levels of As, Cd, and Pb as well as relationship between concentrations of heavy metals in serum and follicular fluid was carried out using student t-test and the significance was set at 0.05.

3 Results

1. Effect of passive smoking on levels of Cd (mg/L), Pb (mg/L) and As (mg/L) in follicular fluid of females undergoing ICSI

It is evident from values shown in Table 1 that passive smoking was associated was significantly (P<0.05) associated with higher concentrations of Cd (mg/L), Pb (mg/L) and As (mg/L) (0.0202±0.0015, 0.0304±0.0029 and 0.0219±0.0021, respectively) in follicular fluid of females undergoing ICSI in comparison with non-smoker females (0.0075±0.0016, 0.0078±0.0024 and 0.0121±0.0013, respectively).

Table 1. Effect of passive smoking on levels of Cd (mg/L), Pb (mg/L) and As (mg/L) in follicular fluid of females undergoing ICSI:

Groups	Heavy metals		
	Cd (mg/L)	Pb (mg/L)	As (mg/L)
Non-smokers (n=28)	0.0075A± 0.0016	0.0078A± 0.0024	0.0121A± 0.0013
Passive smokers (n=47)	0.0202B± 0.0015	0.0304 ± 0.0029	0.0219 ± 0.0021
P value	0.0001	0.0001	0.0024

- Data are presented asmeans ± SE.
-Means having different superscripts in the same column (A,B) differ significantly at P<0.05.

2. Effect of passive smoking on levels of Cd (mg/L), Pb (mg/L) and As (mg/L) in blood serum of females undergoing ICSI

Means of concentrations of heavy metals (Cd, Pb and As) in blood serum of smoker and non-smoker females undergoing ICSI procedure are presented in Table 2. Passive smoking females had significant (P<0.05) increased levels of serum Cd (mg/L), Pb (mg/L) and As (mg/L) (0.0248±0.0021, 0.0375±0.0037 and 0.0373±0.0038, respectively) when compared to non-smokers (0.0127± 0.0026, 0.0107 ±0.0028 and 0.0144±0.0022, respectively).

Table 2. Effect of passive smoking on levels of Cd (mg/L), Pb (mg/L) and As (mg/L) in serum of females undergoing ICSI:

Groups	Heavy metals		
	Cd (mg/L)	Pb(mg/L)	As (mg/L)
Non-smokers (n=28)	0.0127A± 0.0026	0.0107A ± 0.0028	0.0144A± 0.0022
Passive smokers (n=47)	0. 0248 ± 0.0021	0. 0375 ± 0.0037	0.0373 ± 0.0038
P value	0.0006	0.0001	0.0001

- Data are presented asmeans ± SE.
- Means having different superscripts in the same column (A,B) differ significantly at P<0.05.

3. Relationship between concentrations of Cd (mg/L), Pb (mg/L) and As (mg/L) in serum and follicular fluid of females in non-smoker group and passive smoker group undergoing ICSI

As shown in Table 3 , serum concentrations of Cd (mg/L) and Pb (mg/L) (0.0248±0.0019 and 0.0377±0.0037, respectively) in passive smoker females did not differ significantly (P<0.05) from their levels (0.0202±0.0015 and 0.0304±0.0029, respectively) in follicular fluid. However, serum concentrations of As in passive smoker patients (0.0373±0.0038) were significantly (P<0.05) higher than As concentrations in follicular fluid (0.0219±0.0028).

Table 3. Relationship between concentrations of Cd (mg/L), Pb (mg/L) and As (mg/L) in serum and follicular fluid of females in non-smoker group and passive smoker group undergoing ICSI

	Non-smokers (N=28)			Passive smokers (N=47)		
	Cd (mg/L)	Pb (mg/L)	As (mg/L)	Cd (mg/L)	Pb (mg/L)	As (mg/L)
Serum	0.0127A±0.0027	0.0107A±0.0028	0.0144A±0.0022	0.0248A±0.0019	0.0377A±0.0037	0.0373A±0.0038
Follicular Fluid	0.0075A±0.0016	0.0078A±0.0024	0.0121A±0.0013	0.0202A±0.0015	0.0304A±0.0029	0.0219 ±0.0028
P-Value	0.1045	0.4356	0.3614	00679	0.1344	0.0018

- Data are presented as means ± SE.
- Means havingdifferent superscripts in the same column (A, B) differ significantly atP<0.05.

4 Discussion

Cigarette smoke contains Cd and other heavy metals, such as Pb and As, which have effects on reproductive health of animals and humans[12]. The results of the present study revealed the relationship between passive smoking in females and higher serum concentrations of Cd, Pb and As in comparison with non-smoker females undergoing ICSI procedure. In the same respect, mean blood Cd concentrations have been shown to be higher in smokers than in nonsmokers by as much as two- to threefold[13,14]. Smoking is considered an important source for heavy metals intake which is highly concentrated in cigarettes[15,16]. Moreover, active smoking of the father is responsible for passive smoking for the mother[17]. Interestingly, heavy metals could enter the human body through respiratory system[6]. According to[18], the level of exposure to metals in the smoke drawn from a single cigarette is small and likely not acutely toxic, but the accumulation of metals in the body over months, years, and decades of exposure is a health concern. It has been reported that mean Pb levels in the indoor air of homes in which smoking occurs (21.8 ng/m3) was higher compared with levels in homes where no smoking occurs (7.8 ng/m)[19]. Smoking is a major contributor to Pb, Cd, and other metals in the blood[20]. On the other hand, Jung et al.[21] demonstrated a significant association between blood Cd concentration and secondhand smoke exposure in a large nationally representative sample. They found a consistent increase of blood Cd concentration among participants who were exposed to secondhand smoke at workplaces. Additionally, they found that participants who were exposed to secondhand smoke for a longer duration than 1h at home and at total exposure had higher blood cadmium concentration compared with participants who were never exposed to secondhand smoke. However, No difference was observed in lead concentration according to secondhand smoke status.

Follicular fluid microenvironment within the ovarian follicle provides optimal conditions for the maturation and function of both granulosa cells and gametes by virtue of the essential biomolecules[22]. Regarding the concentration of heavy metals in follicular fluid, the present study indicated that heavy metals concentrations in follicular fluid obtained from non-smoker females were significantly higher in follicular fluid obtained from passive smoker females. These results are in agreement with Younglai et al. and Akarsu et al.[23,24] who noted that increasing heavy metals in follicular fluid were suggested to negatively affect the quality of oocytes. Several heavy metals found in tobacco smoke, such as Cd and Pb accumulate in tissues and fluids after smoking[25]. This is a particular issue for Cd and Pb, which have long (10–12 year) half-lives in the human body. Cigarette smoking is a major exposure route for Cd (and to a lesser extent Pb) in the general population[26]. Although the data are controversial and for certain elements scarce, it is known that fertility is impaired in women professionally exposed to Cd, and Pb[27,28]. According to Capcarová[29] the mean level of follicular fluid cadmium was higher in smokers than in non-smokers, and with a dose-effect of smoking. Cd also could accumulate in oocytes of smokers in a dose-dependent manner, in oocytes of cadmium-treated rats. Smoking accelerates ovarian follicular depletion and may lead to diminished ovarian reserve at earlier reproductive ages[30]. Furthermore, passive and active smoking by women had been reported to be associated with delayed conception[31]. Although the CDC[28] reported that humans are exposed to toxic metals in trace concentrations through airborne pollution, and when presented in the blood or follicular fluid, they can have an effect on the reproductive health of women and consequently on the outcome of ICSI procedures[6].

Comparisons between blood serum and follicular fluid indicated no significant differences in concentrations of heavy metals (Cd, Pb and As) in both non-smokers and passive smoker patients undergoing ICSI. However, the result showed significant higher As concentration in blood serum than follicular fluid of passive smoker females. Follicular fluid components are mainly derived from blood besides the locally produced substances[32]. So any changes in blood components are directly reflected in the composition of follicular fluid[33,34]. On the other hand, Bloom et al.[9] also detect moderate positive correlations between concentrations of follicular fluid Cd and blood Cd, although no correlation for follicular fluid Pb and blood Pb. Follicular fluid is a type of blood plasma ultra-filtrate sharing similar yet modified chemical composition[35] and thus anticipated positive correlations between these two compartments for heavy metals was reported previously[36].

Concentrations of elements in follicular fluid of small follicles can differ from those of large follicles. When follicles grow they become filled with fluid of an elemental composition similar to blood. Concentrations of elements in small follicles may represent longer term element exposure, whereas those of growing follicles represent the coincident blood concentrations[36]. The element concentrations within small follicles represent long-term lifestyle exposure of the ovaries, whereas element concentrations in the blood represent shorter exposure that reflects one's recent style of life, this could explain follicular element concentration differences between patients and differences found from blood[36].

5 Conclusion

The present study indicates the results for the effect of passive smoking on levels of Cd, Pb and As in serum and follicular fluid of females undergoing ICSI showing significant higher Cd, Pb and As concentration in passive smokers than non-smokers.

Conflict of interest statement

The authors of this article report no conflict of interest.

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