Chemosphere 135 (2015) 411–417

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Preliminary study of blood methylmercury effects on reproductive hormones and relevant factors among infertile and pregnant women in Taiwan

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HIGHLIGHTS

- 310 infertile and 57 pregnant women were recruited.
- Blood MeHg level was obviously greater in infertile than that in pregnant women.
- Fish and sashimi consumption was the main MeHg exposure source in infertile women.
- Blood MeHg level increased with increasing fish consumption frequencies.
- Eating fish ⩾1 meal/wk got 3–4 fold risk to blood MeHg ⩾5.8 μg/L in infertile women.

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 28 April 2015
Accepted 3 May 2015
Available online 22 May 2015

Keywords:
Methylmercury
Women infertility
Reproductive hormone
Fish consumption

ABSTRACT

Methylmercury (MeHg) is the most poisonous mercury species and an endocrine-disrupting chemical that could cause reproductive and developmental harm effects in animals. In this study, we recruited 310 infertile women and 57 pregnant women and investigated their blood MeHg levels. The distribution of blood reproductive hormone, selenium and zinc levels, and the difference of relevant factors by the reference level of blood MeHg (5.8 μg/L) of infertile women were further examined. Results showed that greater percentages of sashimi consumption, frequencies of Chinese herbal medicine use, alcohol consumption, and lack of physical activity were observed in infertile women than those for pregnant women. Blood MeHg concentration was significantly greater in infertile than that in pregnant women. Significant concentration differences for FSH and LH by the dichotomized reference level of blood MeHg (5.8 μg/L) in infertile women were not observed, which may stem from that these reproductive hormones in participated infertile women were mostly in the normal reference range. Consumption of fish and sashimi represented the major source of MeHg exposure in infertile women. MeHg levels were elevated in infertile women, and consistent with fish consumption frequency. Compared to the referent level of blood MeHg levels <5.8 μg/L, the elevated blood MeHg levels (⩾5.8 μg/L) in infertile women were 3.35 and 4.42 folds risk in categorized frequencies of fish consumption 1–2 meals per week and more than 3 meals per week, respectively. The obtained results provide evidences and help updating the advisory of fish consumption and improving women's reproductive health.

1. Introduction

Recently, environmental contamination due to rapid economic growth and increasing population is greatly concerned in Asian developing countries (Agusa et al., 2007). Some studies postulated that mercury (Hg) may influence physiological levels of reproductive hormones (Iavicoli et al., 2009). Agusa et al. (2007) studied 20

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http://dx.doi.org/10.1016/j.chemosphere.2015.05.006
0045-6535/

subjects (5 males and 15 females) from Cambodia with Hg concentrations in blood of 5.2 ± 58 μg/L and reported an association between Hg exposure in humans and serum hormone. The analytical results also indicated that serum estrone and estradiol levels were positively correlated with blood Hg levels for both males and females, indicating possible induction of female hormones by Hg exposure (Agusa et al., 2007). Higher blood Hg concentration was shown to associate with female infertility in a study of Hong Kong (Choy et al., 2002). The study also indicated that Hg in the environment may interfere with female endocrine system and cause reproductive harms.

Methylmercury (MeHg) is the most poisonous Hg species. MeHg readily bioaccumulates in exposed organisms and has a longer half-life causing severe harm to the human body (Hong et al., 2012). Although the adverse reproductive effects from MeHg exposure to fertility of women have not been fully elucidated, toxicological studies have provided some insights (Murata et al., 2011; Tsuda et al., 2011; Hong et al., 2012). Strong evidences showed that MeHg is a endocrine-disrupting chemical that could cause reproductive and developmental harm effects in mammals, birds, reptiles, fish, mollusks, and others (Adams and Frederick, 2008; Frederick and Jayasena, 2011; Jayasena et al., 2011). The impacts of MeHg exposure on reproductive toxicology of female animals have been well reported, but studies on the reproductive hormone and relevant factors of infertile women have not been conducted thus far. The essential metals, such as selenium (Se) or zinc (Zn), have shown to protect against the intoxication of MeHg (Chapman and Chan, 2000; Ralston et al., 2008; Ralston and Raymond, 2010). As aforementioned, reproductive hormones imbalance, current smoking, essential metals, and obesity were associated with the risk of infertility in women of reproductive age (Augood et al., 1998; Kelly-Weeder and Cox, 2006). The present study aimed to investigate the blood MeHg distribution in infertile and pregnant women. The blood reproductive hormone (i.e., FSH, LH, and PRL) and essential metal (Se and Zn) levels for infertile women cut off by the reference level of blood MeHg (5.8 μg/L) exposure were compared. Furthermore, we evaluated the adjusted odds ratios (aOR) of elevated blood MeHg levels and relevant variables in infertile women by logistic regression model. The obtained results are crucial to clarify the relationship on MeHg levels among reproductive hormone, and relevant factors of infertile women. A better insight into the effects of MeHg exposure on infertile women can thus be provided in this study.

2. Materials and methods

2.1. Study subjects

All enrolled 367 women who attended the Department of Obstetrics and Gynecology in the Taiwan Adventist Hospital from August 2008 to March 2010 were between 18 and 45 years old. No participants had diagnosis of congenital adrenal hyperplasia or Cushing's syndrome in this study.

A structural questionnaire was administered to each study subject face-to-face by a trained interviewer to obtain sociodemographic information, lifestyle characteristics, and menstruation history.

The present study was conducted in accordance with the guidelines of the Institutional Review Board of Taipei Medical University (Approval number: P950045) and the Taiwan Adventist Hospital Investigational Review Board (TAIRB number: 989801A). All subjects provided written informed consent before beginning the study.

2.1.1. Infertile women

The definition of infertile women were as presenting a complaint of difficulty conceiving after 1 year of normal sexual activity with the intention to become pregnant (Cooper et al., 2010; Gurunath et al., 2011).

As a result of polycystic ovary syndrome (PCOS) is one of the mainly causes of female subfertility (Azziz et al., 2004) and a prevalent endocrine disorder in women, it might interfere with our observation on hormones and blood MeHg. Therefore, infertile women with a previous diagnosis of PCOS were excluded from this study. The diagnosis of PCOS was based on women who revealed symptoms of chronic anovulation associated with clinical or biochemical hyperandrogenism. Patients with diabetes mellitus, nonclassical adrenal hyperplasia, 21-hydroxylase deficiency, hyperprolactinemia, androgen-secreting tumors, hypothyroidism, and receiving hormonal therapy were all excluded from this study (Ehrmann et al., 1999; Legro et al., 1999; BCJM, 2004). There were 310 infertile women recruited in our study.

2.1.2. Pregnant women

All eligible women underwent an ultrasound examination and fetal heartbeat assessment to confirm pregnancy and all of those pregnant participants had a safe delivery. Women who had received artificial insemination or *in vitro* fertilization (IVF) were included. Eligible pregnant women were defined as successful conception in one year with normal sexual activity. There were a total of 57 pregnant women consented to participate this study.

2.2. Determination of blood metal concentrations

2.2.1. Determination of methylmercury concentrations

Blood MeHg was analyzed according to U.S. Environmental Protection Agency Method 1630 (U.S. EPA, 2001). Briefly, 0.1 mL sample and 2 mL methanol (25% KOH) were added into Teflon bottle. The sample was decomposed using a heating block set at 75 °C, and then 10 mL dichloromethane (DCM) and 2 mL HCl were added. DCM was removed by a Brooks Rand MeHg distillation system set at 70 °C. The sample was diluted to 100 mL. 5 g diluted sample, 0.3 mL acetate buffer, and 0.04 mL sodium tetraethylborate were added to a brown vial with 30 mL deionized water. The preprocessed sample was purged with N_2 and moved to a Tenax trap. The absorbed trap was dried with N_2, and then desorbed through heating. The sample was removed from the trap, subjected to a MERX system (Brooks Rand Co, Seattle, WA, USA) with cold vapor atomic fluorescence spectrophotometry as detector for analysis (Kim et al., 2012).

Toxic Metals in Caprine Blood (955c) as the certified reference material from the National Institute of Standards and Technology, trace elements whole blood level 3, was used to perform a standard material test to ensure the precision and accuracy of the blood metals analyses. The precision and accuracy levels of MeHg were measured 97.6% and 99.9%, respectively.

2.2.2. Determination of selenium and zinc concentrations

Blood samples were collected from each participant in 10 mL EDTA tubes. Approximately 1 mL of each whole blood sample was digested (CEM, Model MDS-2000) with 3 mL 65% nitric acid (Suprapur, Merck). After cooling, the residual fluid was diluted to 50 mL with deionized water (18 MΩ), and then filtered with 0.45 μm filtered tap. The levels of Se and Zn were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Thermo X-series II). Each of the samples was analyzed in triplicates. The detection limit for Se and Zn was 0.49 and 0.37 μg/L, respectively. The standard reference material, trace elements whole blood level 2 (Seronorm™; SERO, Billingstad, Norway), was used to ensure the precision and accuracy of the blood essential metals analyses. The