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Low Serum Selenium Levels in Iranian Women with Idiopathic Primary Ovarian Insufficiency: A Case-Control Study

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Abstract

Aim: This study aimed to evaluate serum selenium levels and glutathione peroxidases (GPxs) activity in women with idiopathic primary ovarian insufficiency (iPOI) and in healthy fertile women. Materials and methods: This case-control study was conducted in Al-Zahra Hospital affiliated to Tabriz University of Medical Sciences, Tabriz, Iran. The iPOI group consisted of 32 infertile women with iPOI (with amenorrhea/oligomenorrhea at least for the last 4 months and FSH>40 mIU/ml) . The control group consisted of 31 age-and BMI-matched healthy fertile women. In the all participants, serum selenium levels were measured by an atomic absorption spectrophotometer, and plasma GPx activity was measured by a Glutathione Peroxidase Activity Assay Kit after about 12 hours of fasting. Results: There was a significant lower serum selenium levels in the iPOI group compared to the control group (Adjusted Mean Difference (AMD) =-15.1 µg/ml, 95% CI: -24.8 to -5.3). The plasma GPx activity was lower in the iPOI group compared to the control group, although not significantly (AMD =-67.0 U/ml, 95%CI: -194.5 to 60.3). Both groups had similar selenium dietary intake and in accordance with the recommended dietary allowance (RDA) of 55 µg/day. Conclusion: This study showed a significant decline in the serum selenium levels of women with iPOI. To determine their selenium-dependent antioxidant defence capability, as measured by GPx activity, more large-scale studies are required.

Key words: Primary ovarian insufficiency, infertility, selenuim, glutathione peroxidase, antioxidant.

Introduction

Primary ovarian insufficiency (POI) is the cessation of ovarian function in women under the age of 40 and is identified by amenorrhea or oligomenorrhea at least for 4 months, hypergonadotropism and hypoestrogenism. It affects 1/100 women under the age of 40 and is responsible for about 10% of female infertility with ovulatory dysfunction. Besides infertility women with POI are confronted to an increased risk of cardiovascular diseases, osteoprosis and earlier death. It seems that the term POI is more accurate than POF (premature ovarian faluire) to explain the problem since the course can be flactuating, reversible and time-consuming. Elevated levels of folicule stimulating hormon (FSH) more than 40 mIU/ml is indicative of POI. Several factors have been described for premature depletion of the primordial follicule reserve, although for most of the cases the causative factor remains unknown. These women are diagnosed with

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idiopathic POI (iPOI). (Ebrahimi and Asbagh, 2011; Coulam *et al.*, 1986; Tucker *et al.*, 2016; Welt, 2008; De Vos *et al.*, 2010)

It has been suggested that oxidative stress plays a role in germ cells apopetosis. (Limoli *et al.*, 1998) Oxidative stress affects oocytes through reactive oxygen species (ROS). Although ROS, produced in the ovaries, is important for some physiologic process, higher-than-optimal levels of ROS induce oogenesis damage and lead to low oocyte count, so risk of some ovarian disease such as POI increases. (Kumar *et al.*, 2012; Agarwal *et al.*, 2005)

Selenium (Se), an essential trace element, presents in the active center of glutathione peroxidase enzymes (GPxs). These Se-dependent antioxidant enzymes contribute to neutralization, elimination and prevention of ROS production. (Pieczyńska and Grajeta, 2015) Se serves several other functions in human general and reproductive health such as anti-inflammatory activities and fertility promotion. (Pieczyńska and Grajeta, 2015; Mistry *et al.*, 2012) Normal serum Se level range for adult non pregnant women is (63 – 160 µg/l). (Abbassi-Ghanavati *et al.*, 2009) Although a diet poor in Se is the main reason for Se deficiency, disorders in Se bioavailibility process and also some active disease yield it. The decline in Se levels potentially causes health problems especialy pertained to chronic diseases, cardiovascular diseases and infertility. (Pieczyńska and Grajeta, 2015 ;Mistry *et al.*, 2012) Further more Selenoprotein enzymes, such as GPxs, activity may negatively be affected. (Bleys *et al.*, 2007)

To the best of our knowledge, there are no data on the serum Se level, the GPxs activity and the mean daily selenium intake in infertile women due to iPOI. The present study was therefore conducted to assess and compare them to those in healthy fertile women. The results can provide a valuable data and contribute to the current knowledge about POI pathogenesis

Methods

Study type and participants

The present case-control study was conducted between August 2017 and August 2018 in Al-Zahra Hospital, affiliated to Tabriz University of Medical Sciences, Tabriz, Iran. Institutional Review Board at Tabriz University of Medical Sciences approved the study (IR.TBZMED.REC.1396.226). The iPOI group consisted of 32 women who were referred to the infertility clinic and diagnosed with iPOI. The control group consisted of 31 healthy and fertile women who were referred to the gynecology clinic (with vaginitis or non-inflammatory diseases). All participants gave Written Informed Consent to take part in the study.

The eligibility criteria for participants included: reading and writing literacy, the ability to fill out the 24-hour food record, residing at Tabriz for at least the last six months, non-smoker, non-vegetarian and BMI <30.

Selection criteria

The iPOI group inclusion criteria were: women aged 20-40 years with amenorrhea or oligomenorrhea during at least the last four months and FSH >40 mIU/ml, recorded as at least 2 readings 1 month apart, no any known causes of POI (chromosomal or genetic disorders; auto immunologic, iatrogenic, infection causes; and environmental toxins). The control group criteria were age and body mass index (BMI) matched healthy fertile women with regular 22-35-day menstruation cycles (with changes ≤2 days) over at least the last 3 months; having had at least one child, no history of infertility or POI; non pregnant, non-breastfeeding, non IUD user.

Women with cardiovascular diseases; hypertension; gastrointestinal diseases; thyroid disorders; renal diseases; cancer; diabetes; obesity; chronic inflammatory diseases such as rheumatoid arthritis; endometriosis (endometriuma); uterine fibromas, ovarian infections; polycystic ovaries; a history of chemotherapy, radiotherapy and ovarian surgery or oophorectomy were excluded from the study. We excluded women who have been using contraceptive pills, estrogens, progesterones, ovulation stimulants over at least the last three months, also multivitamins, minerals or any supplements containing selenium or herbal medications or have been on specific diet over at least the last three months. The main objective of the study was to compare infertile women due to iPOI to healthy fertile women in terms of serum selenium levels and glutathione peroxidase activity, and the secondary objective was to compare the nutritional status of these two groups in terms of selenium intake.

Sample size

The total sample size estimation was 62 participants using G^* POWER (version 3.1.2.). According to the Rafraf *et al.* study on serum selenium level in healthy premenopausal women and considering m_1=77.72 m2=66 (with presumption of lower serum selenium level in infertile iPOI patients by 15%), Sd_1=Sd_2=16.92 and power of 85%, the number of each group was obtained as 31. According to the Ha *et al.* study on glutathione peroxidase (GPx) activity in healthy premenopausal women with presumption of lower level of GPx activity in infertile iPOI patients by 20% and considering m_1=4.72 m_2=3.94, Sd_1=Sd_2=0.93 and power of 85% the number of each group obtained as 31.

Data collection

Two visits were arranged for each participant during the study. During the first visit, the participants completed the demographic-anthropometric-obstetric questionnaire. The important parameters enquired were: age, BMI, menarche age, gravidity, parity, activity level (householding, householding plus less than 150 minutes exercise per week [exercise], householding plus more than 150 minutes exercise per week [heavy exercise]), job, education, income. Height and weight were measured by FAZZINI stadiometer (an adult scale of height; RODO code: S7200HR, made in Italy). The Body Mass Index (BMI) was calculated as the ratio of weight in kilograms, divided by height in meters squared. All the measurements were recorded in the relevant part of the questionnaire. For the second visit, the iPOI group participants returned within one week after the first visit. The control group participants returned during their early follicular stage (i.e. the second or third day of menstruation, a time when FSH would be at its highest level during the menstrual cycle). During the second visit, 5-cc blood samples were taken from each participant following 12 hours fasting and collected into two tubes, one with no anticoagulants to determine serum selenium levels and the other containing anticoagulant (K2 EDTA) to measure glutathione peroxidase activity. First serum FSH levels of the participants were investigated. Then the blood samples were centrifuged at 3000 rpm for 10 minutes and were kept at -70 °C until analysis. The selenium concentration was measured by CTA 3000 device (made in the UK) using atomic absorption spectrophotometry by hydride ion production in the range of 3-750 µg/L. The glutathione peroxidase activity in the plasma was measured by ZellBio (GmbH, Ulm, Germany), a Glutathione Peroxidase Activity Assay Kit based on colorimetric assessment using microplate reader at 412 nm and according to the manufacturer's instructions.

Nutrition Status

During the first visit, the 24-hour food record forms were given to the all participants to carefully record all their routine food and drink consumed for 3 days (two work days and one weekend day). Within the second visit, the filled out 24-hour food record forms were delivered and any ambiguity in their registered foods was clarified in person. The three-day 24-hour nutrition registration data were analyzed in Nutritionist-IV software (First Databank, San Bruno, CA, USA) modified for Persian foods in terms of calorie, carbohydrate, fat, protein and selenium intake and the mean nutrition intake over this period was measured.

Data analysis

Data were analyzed in SPSS-21. The normality of the distribution of the quantitative data was confirmed using the Kolmogorov-Smirnov test. Statistical analysis included independent t-test Chi-square, and Pearson`s. The mean serum Se levels and glutathione peroxidase activity were compared using the independent t-test in the bivariate analysis and using the general linear regression in the multivariate analysis with the confounding factors controlled. $P<0.05$ was taken as significant.

Results

A total of 63 women (32 in the iPOI group and 31 healthy fertile women age-and BMI-matched in the control group) were included in the study. Table 1 presents mean age, BMI, menarche age, FSH, obstetric history, activity level, education, job and incomes. The iPOI and control groups were significantly different in certain obstetric features: gravidity and parity were significantly lower in the iPOI group compared to the controls ($P<0.001$). There were significant differences between the two groups in FSH concentration ($P<0.001$), and FSH was significantly higher in the iPOI group compared to the controls. No significant differences were found between the two groups in the level of activity ($P=0.352$); however, there were significant differences between them in terms of job and education ($P<0.05$).

Serum Se levels were significantly lower in the iPOI group compared to the control. The adjusted Mean Difference (aMD) was -15.1 µg/ml (95% CI: -24.8 to -5.3) and was adjusted for calorie, fat, selenium, activity level, education and job. The plasma GPx activity was lower in the iPOI group compared to the control group, but not significantly (aMD= -67.0 U/ml, 95% CI: -194.5 to 60.3); adjusted for calorie, fat, selenium, activity level, education and job (Table 2).

The nutrition status in terms of calorie, carbohydrate, fat and selenium intake was the same in the both groups ($P>0.05$) (Table 3).

Regarding all participants as one group (combined group, n=63), a negative correlation was found between Se and FSH (p=.001) (Table 4).

Discussion

Our main findings were significantly lower levels of selenium in iPOI group compared to those in the control group with no difference in mean daily selenium intake, and a negative correlation between Se and FSH.

A capable antioxidant defense system protects cells against ROS harmful effects by overwhelming oxidative stress, but otherwise the risk of aging, cell-death and the development of some chronic diseases increase. (Finkel and Holbrook, 2000) Oxidative stress and inflammatory process may have a pathogenic role in iPOI. It has been suggested that for many women diagnosed with iPOI there could be a basis in autoimmunity and/or inflammation. (Kumar *et al.* 2012; Weiss *et al.*, 2009; Dragojević-Dikić *et al.*, 2010) In the ovaries oxidative stress, due to supra-optimal levels of ROS, impairs nuclear and mitochondrial DNA, and results in increased apoptosis. Along to this, a mass amount of lipid peroxide products could also transform protein structure and function and induce inflammatory cytokines chemotaxicity. (Kumar *et al.* 2012; Kumar *et al.* 2010) Some studies have shown decreased Se levels in inflammatory response. (Renko *et al.*, 2009; Nichol *et al.*, 1998) Thus, the lower serum Se levels assessed in the present study could be due to the effects of the inflammation of iPOI on Se concentration.

There is scares data in the literature on oxidant-antioxidant status of women with iPOI, also there is no data on the Se status of these women. Studies conducted on other ovarian disease such as poly cystic ovarian syndrome (Coskun *et al.*, 2013) or pregnancy-related complications such as preterm labor, preterm premature rapture of membranes (Rayman *et al.*, 2011; Iranpour *et al.*, 2009) and preeclampsia (Mahomed *et al.*, 2000; Mistry *et al.*, 2008) have found low serum/plasma Se concentrations and linked it to the increased oxidative stress and inflammatory response. The result of our study was similar to the findings of those studies. Our result was not in accordance with the studies that have suggested no decrease in selenium status following menopause at normal age. (Ha and Smith, 2009; Rafraf *et al.*, 2008) This difference may be related to the changes in Se hemostasis due to increased oxidative stress in iPOI women.

The present study showed no significantly change of plasma GPx activity in both groups, despite lower Se concentration in iPOI group compared with the control. This finding might be a result of the effects of the small sample size or other factors, not measured in our study, which influenced the GPx antioxidant activity. It did not allow to draw a precise conclusion. GPxs antioxidants play key roles in reducing the toxic effects of hydrogen peroxide (H_2O_2) and lipid peroxide by converting them into harmless products such as water and alcohol. They also are involved in maintaining the integrity of the cell membrane, supporting the production of prostacyclins, preventing the harmful effects of ROS on lipids, lipoproteins and ribonucleic acid (DNA). (Mistry *et al.*, 2012; Kieliszek and Błażejak, 2016)

In our study a negative correlation was found between Se and FSH. Sparse studies have been conducted to investigate the relationship between gonadotropins and/or female sex steroids and Se status, and not all of them found a correlation. Ha *et al.* observed a positive association between estrogen and selenium status in healthy women of reproductive age. (Ha and Smith, 2003) Considering the negative feedback between estrogen and FSH during menstrual cycle, our finding was in accordance with their result.

The mean daily Se intake among women participated in our study was not different and it was almost in accordance with the current recommended diatery allowance RDA of 55 µg/day. (Mistry *et al.*, 2012)

The European Food Safety Authority (EFSA) suggested 70 µg selenium/day. (EFSA, 2014) The tolerable upper intake limit (UL) is estimated at 400-700 µg selenium/day. (Kieliszek and Błażejak, 2016) Human body Se status depends on food supplies. Ha *et al.* (2009) reported good Se status (105 µg/l) of American healthy adult women with a dietary intake of Se greater than the RDA (90 µg/day). Furthermore, to attain maximum GPxs expression it has been suggested that serum/plasma Se levels at almost 90-100 µg/l (Alfthan *et al.*, 1991; Duffield *et al.*, 1999). Razavi *et al.* (2016) reported that Se supplementations for 8 weeks among PCOS women improved their reproductive outcomes. In the view that there is a delicate border between the essential and the toxic amount of Se, (Navarro-Alarcon and López-Martınez, 2000) whether iPOI women benefit from consuming additional amount of Se (in the form of supplements or food fortification), farther studies will reveal.

Conclusion: the present study showed low serum Se levels of women with iPOI and a negative correlation between Se and FSH. The important controversial issue is the possibility of a cause-and-effect relationship between Se concentrations and iPOI in these women. Epidemiological studies are warranted to clarify the issue. To evaluate the Se-dependent antioxidant defence capability, as measured by GPxs activity, in iPOI women more large-scale studies are required.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.

The present study has been conducted as an MSc thesis at the School of Nursing and Midwifery of Tabriz University of Medical Sciences.

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Table 1: Characteristics of participants by study groups

Characteristics	iPOI N=32 Mean ± SD	Control N=31 Mean ± SD	P-value
Age (years)	35.6 (4.1)	35.9 (5.6)	.802†
BMI (kg/m^2)	25.5 (3.0)	25.5 (2.1)	.989†
Menarche age (years)	13.2 (1.6)	12.7 (1.1)	.192†
FSH (mIU/ml)	74.2 (32.9)	6.3 (2.3)	.001†
Gravidity*			.001‡
Nulligravida	17 (53.1)	0 (0.0)	
Gravida 1	9 (28.1)	17 (54.8)	
Multigravida	6 (18.8)	14 (45.2)	
Parity*			.001‡
Nullipara	18 (56.3)	0 (0.0)	
Para 1	12 (37.5)	18 (58.1)	
Multipara	2 (6.3)	13 (41.9)	
Activity*			.352‡
Householding	9 (28.1)	8 (25.8)	
Exercise	20 (62.5)	16 (51.6)	
Heavy exercise	3 (9.4)	7 (22.6)	
Education*			.037£
Primary-Secondary	11 (34.4)	4 (12.9)	
High school	11 (34.4)	11 (35.5)	
University	10 (31.3)	16 (51.6)	
Job*			.006‡
House wife	21 (65.6)	8 (25.8)	
Household jobs	6 (18.8)	11 (35.5)	
Employed	5 (15.6)	12 (38.7)	
Incomes*			.051£
Low	10 (31.3)	3 (9.7)	
Modrate	19 (59.4)	23 (74.2)	
High	3 (9.4)	5 (16.1)	

Note: iPOI: idiopathic primary ovarian insufficiency. BMI: weight (kg)/ height (m)^2. FSH: Follicul stimulating hormon. SD: standard diviation.
Variables indicated with * are as number (%).
† Independent t student.
‡ Chi-Square.
£ Chi-Square for trend

Table 2: Selenium levels and gluthathione peroxidas (GPx) activity in participants by study groups

Serum/plasma	iPOI n=32 Mean ± SD	Control n=31 Mean ± SD	aMD* (95% CI)	P-value
Selenium (µg/L)	60.7 (17/8)	78.1 (14.1)	-15.1 (-24.8 to -5.3)	.001**
Glutathione peroxidase (U/ml)	825.5 (208.0)	847.6 (227.1)	-67.0 (-194.5 to 60.3)	.296**

Note: iPOI: idiopathic primary ovarian insufficiency. SD: Standard Deviation
* adjusted mean difference, adjusted for calorie, fat, dietary selenium, education, job
** multi variable linear regression

Table 3: Dietray estimates for daily intake of selected nutrients by study groups

	iPOI n=32 Mean ± SD	Control n=31 Mean ± SD	P-value
Calorie (kcal)	1532.0 (653.1)	1311.8 (719.2)	.208
Carbohydrate (g)	217.5 (116.6)	176.0 (94.9)	.127
Protein (g)	63.0 (31.3)	50.4 (24.0)	.076
Fat (g)	48.0 (21.3)	48.2 (30.1)	.974
Selenium (µg)	59.7 (29.7)	57.3 (27.7)	.743

Note: iPOI: idiopathic primary ovarian insufficiency. SD: Standard Deviation.

Table 4: Correlation of serum Se with serum FSH

Combined group (n=63)	Se	
	r	p
FSH	-.424	.001
r: correlation coefficient		