Correction to Reduced Serum Selenium Concentration in Miscarriage Incidence of Indonesian Subjects

by Herlambang Herlambang

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CORRECTION

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The original version of this article unfortunately contained a mistake. The name of "Herlambang herlambang" is now corrected in the author group of this article.

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Reduced Serum Selenium Concentration in Miscarriage Incidence of Indonesian Subjects

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Abstract Selenium is an essential nutrient for human health, and maternal selenium concentration has been reported to be associated with pregnancy outcome. To further investigate the possible role of selenium (Se) in miscarriage, we conducted a case–control study to evaluate the correlations among selenium status, glutathione peroxidase activity, and spontaneous abortion. A total of 46 subjects with normal pregnancies and 25 subjects with spontaneous abortion were recruited, and their serum selenium concentrations and serum glutathione peroxidase activities were analyzed. The total serum selenium concentrations in subjects with normal pregnancies were significantly higher than those of subjects with spontaneous abortion; however, the glutathione peroxidase activities were similar in both groups. We further separated the subjects into smoking and nonsmoking groups, and the logistic regression analysis suggested that total serum selenium concentration, but not serum glutathione peroxidase activity or smoking, was significantly correlated with the incidence of miscarriage. The present study thus reaffirms that low serum selenium levels are associated with miscarriage and that selenium plays an important role in pregnancy maintenance.

Keywords Selenium · Glutathione peroxidase · Antioxidant · Pregnancy · Miscarriage

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Introduction

Miscarriage, also commonly referred to as spontaneous abortion, is defined as a pregnancy that ends spontaneously before 20 weeks of gestation. Miscarriage is estimated to occur in 12–15 % of clinical pregnancies and 17–22 % of all pregnancies (including early pregnancy losses) [1]. Spontaneous abortion can be caused by many factors, including oxidative stress [2–4]. Oxidative stress is a condition caused by an imbalance of free radical production and the body's antioxidant defense system. One enzyme that serves as a primary antioxidant defense factor in humans is glutathione peroxidase (GPx). The main role of GPx is to maintain appropriately low levels of hydrogen peroxide within the cell, thus decreasing the potential for damage to membranes and other cell structures [5]. GPx is a selenium (Se)-dependent enzyme, and Se is incorporated into its active site [6, 7]. Thus, the concentration of Se in the body, which depends on dietary intake, influences the levels of GPx and other Se-dependent antioxidant enzymes that function to protect the body against free radicals.

Se is an essential trace element, and Se deficiency is associated with the development of several diseases [8–12]. The nutritional benefits of Se are believed to be related to its role as an important component of several antioxidant enzymes that protect cells against the effects of

free radicals [13]. Apart from its protective role in cancer and cardiovascular diseases [13], Se has also been reported as an essential nutrient in pregnancy, and maternal Se concentration has been reported to be associated with reproductive performance, including infertility, preeclampsia, early childhood wheezing symptoms, miscarriage, and retained placenta [14–17]. The relationship between Se and miscarriage, however, has not been determined conclusively [18]. The levels of free radicals and reactive oxygen species are high during miscarriage [19]. Therefore, Se, as an important component of the antioxidant enzyme GPx, may play a significant role in the development of miscarriage.

In this study, we compared the serum Se concentrations and GPx activities of subjects with spontaneous abortion with those of subjects with normal pregnancies to further confirm the possible role of Se in miscarriage.

Methods

Study Design

We conducted a case–control study including 46 subjects with normal pregnancies and 25 subjects with spontaneous miscarriage. All of the subjects were under 35 years old and were evaluated between 8 and 20 weeks of gestation. Normal pregnancies were confirmed using ultrasonography (USG). Subjects with incomplete spontaneous abortion were included in the miscarriage group. All subjects were confirmed to be free from any major congenital disorders, uterine disorders, or malignancies using USG. No subjects were clinically diagnosed with heart disease, kidney disorders, hypertension, or diabetes mellitus. A more detailed summary of the subject characteristics is shown in Table 1.

Blood samples were collected from subjects via antecubital vein puncture. All samples were then centrifuged to separate the plasma and serum. After centrifugation, the serum samples were frozen at −80 °C for subsequent analysis of total Se concentration, GPx activity, and other standard blood parameters such as hemoglobin, leucocytes, thrombocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, glucose, activated partial thromboplastin time, and prothrombin. The study protocol was approved by the Universitas Padjadjaran Ethics Committee, and all subjects provided informed consent prior to participation.

Se Concentration

The Se concentration was calculated based on fluorometric measurements at an excitation wavelength of 378 nm and an emission wavelength of 525 nm to determine the concentration of piazselenol, which is produced by the reaction of selenite

Table 1 Characteristics of the study participants

	Control (n=46)	Miscarriage (n=25)	p value
Pregnancy characteristics			
Age (years)	28.72±4.62	28.40±4.10	ns
Height (cm)	154.34±5.87	152.88±4.61	ns
Weight (kg)	54.72±9.32	60.84±9.41	0.018
Gestational age (weeks)	11.13±1.91	10.80±1.80	ns
Gravidity	2.31±0.69	2.32±0.69	ns
Parity	1.09±0.78	0.72±0.74	ns
Lifestyle characteristics			
No. of smokers	18	11	
No. of alcohol consumers	2	6	
Blood characteristics			
Hemoglobin (g/dl)	10.61±0.70	10.08±0.40	0.001
Leucocyte (mm^3)	7,050±1,574	7,392±1,798	ns
Thrombocyte (mm^3)	184,875±31,175	186,920±31,395	ns
Hematocrit (%)	40.50±4.57	39.76±4.67	ns
MCV (fl)	85.54±3.30	85.89±3.65	ns
MCH (pg)	28.52±1.10	28.65±1.24	ns
MCHC (g/dl)	33.83±1.12	33.89±1.03	ns
Glucose (mg/dl)	87.69±10.88	90.56±12.73	ns
APTT (s)	31.33±3.09	30.03±4.01	ns
Prothrombin (s)	9.73±0.65	9.62±0.66	ns

ns not significant

with 2,3-diaminonaphtalene [20]. The validity of the selenium analysis was confirmed by the measurement of a reference material (bovine liver, SRM 1577b, National Institute of Standards and Technology, USA).

GPx Activity

GPx activity was measured spectrophotometrically based on the quantity of NADPH used in the reduction of glutathione, which was generated from oxidized glutathione through a reaction catalyzed by glutathione reductase [21]. Hydrogen peroxide and reduced glutathione were used as substrates at 37 °C in the presence of NADPH and glutathione reductase. The rate of NADPH oxidation at 340 nm was used as an index of hydrogen peroxide reduction.

Statistical Analysis

Paired *t* tests were used to compare the total selenium concentrations and GPx activities of the miscarriage and control groups. Further logistic regression analysis was performed using R statistical software [22] to determine whether Se concentration, GPx activity, or smoking habits were related to pregnancy outcome. A *p* value of less than 0.05 was considered statistically significant.

Results

The total Se concentrations in the control and miscarriage groups are shown in Fig. 1a. The Se concentration in the miscarriage group (66.71±13.55 ng/ml) was significantly lower ($p=0.023$) than that of the control group (76.36±18.22 ng/ml). Meanwhile, as shown in Fig. 1b, the GPx activity of the miscarriage group (135.27±24.33 Δmmol NADPH/min/l) did not differ significantly from that of the control group (130.17±24.89 Δmmol NADPH/min/l).

Due to the large number of smokers in both the miscarriage and control groups, we further separated and analyzed the effect of smoking in each group. As shown in Fig. 2a, there were no significant differences in the Se concentrations of nonsmoking subjects in the miscarriage (75.17±17.53 ng/ml) and control (78.21±19.62 ng/ml) groups. However, among the smoking subjects, the Se concentration in the miscarriage group (59.90±12.96 ng/ml) was significantly lower ($p=0.022$) than that in the

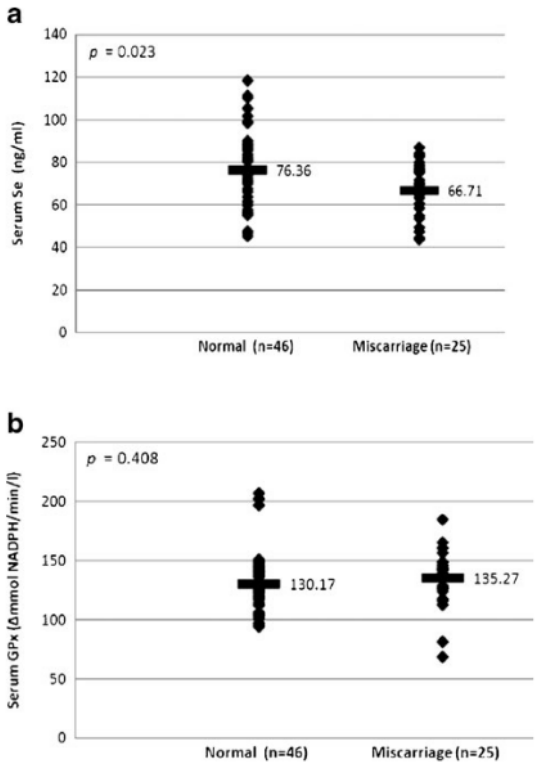

Fig. 1 Serum Se concentration (**a**) and serum GPx activities (**b**) in subjects with normal pregnancies and miscarriage

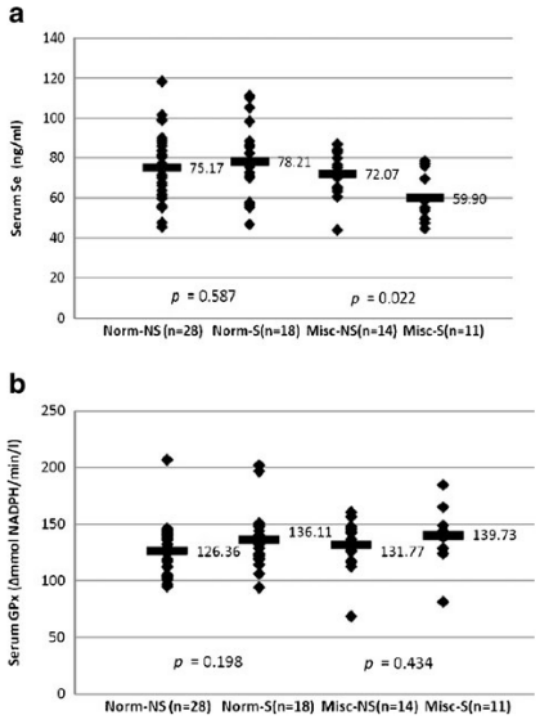

Fig. 2 The effect of smoking on serum Se concentration (**a**) and serum GPx activities (**b**) in the normal and miscarriage groups. *Norm-NS* normal pregnancy group, nonsmoking; *Norm-S* normal pregnancy group, smoking; *Misc-NS* miscarriage group, nonsmoking; *Misc-S* miscarriage group, smoking

control group (72.07±11.82 ng/ml). There were no significant differences in GPx activity between the miscarriage and control groups in either the nonsmoking or smoking subjects (Fig. 2b).

The correlation between Se concentration and GPx activity was also analyzed using logistic regression analysis. In the nonsmoking subjects, Se concentration was positively correlated with GPx activity (p=0.002). However, this correlation was not found in the smoking subjects (p=0.621).

Discussion

The results of the present study showed that the women in the miscarriage group had significantly lower Se concentrations than did the women in the control group. The decreased Se in women with miscarriage may increase the free radicals and oxidative stress.

Oxidative stress has been suggested to play a role in early miscarriage. Jauniaux and co-workers have reported that premature oxygenation in the early embryonic environment may result in early pregnancy loss. In their study, oxygenation was accompanied by an induction of antioxidant defense precursors, including catalase, GPx, and superoxide dismutase [23]. Previous research has also demonstrated an increase in oxidative stress markers in the placental tissue of women with early pregnancy losses compared with controls and suggested that this increase may be due to the premature establishment of maternal placental perfusion [24]. Increased oxidative stress may also alter the placental vasculature, leading to early miscarriage [25].

The loss of antioxidant defenses has also been reported as an important cause of recurrent miscarriage [26]. Lipid peroxidation products, which are biochemical markers of oxidative stress-induced membrane damage, reached high levels immediately before spontaneous abortion [27].

Placental oxidative stress may also impair placental development or syncytiotrophoblast degeneration in early pregnancy and thus may affect pregnancy outcomes, including miscarriage and preeclampsia [28]. Oxidative damage to the trophoblast is reported as a key factor in early pregnancy loss [29]. Thus, oxidant and antioxidant imbalance likely play a role in miscarriage [30].

During pregnancy, metabolic demands increase due to the changes in maternal physiology and the requirements of the growing fetus [31]. Insufficiencies of certain essential vitamins and micronutrients may induce biological competition between the mother and conceptus, which will affect their health status [32]. Deficiencies of specific trace elements, including Se, during pregnancy are associated with neonatal mortality and morbidity [33, 34].

Se has been implicated in pregnancy outcomes [35–38], and it plays a role in the immune response and resistance to infection [39]. At least three mechanisms have been proposed to explain the association between miscarriage and Se deficiency. The first is the loss of Se-dependent antioxidant capacities, leading to damage of biological membranes and DNA [36]; the second is reduced antithrombin III activity due to Se deficiency [40]; and the third is the reduced activity of selenoenzymes (enzymes containing Se), which are reported to downregulate the expression of pro-inflammatory genes associated with adverse pregnancy outcomes [39, 41, 42].

In this study, the GPx activities of both groups were similar. This observation may be due to the fact that Se-dependent antioxidants are not only GPx enzymes. As reviewed in our previous study, Se is a component of several important antioxidant enzymes, including GPx and thioredoxin reductase (Trx). The major physiological role of GPx is to maintain appropriately low levels of hydrogen peroxide within the cell, thus decreasing the potential for damage from free radicals. It provides a second line of defense against hydrogen peroxide, which can damage membranes and DNA. Trx is a widely distributed redox protein that regulates several intracellular redox-dependent processes and is involved in repair mechanisms that are essential for DNA synthesis [13].

In addition, in early pregnancy, trophoblasts are released, allowing the onset of placental circulation. The release of trophoblast reduces the flow of blood into the intervillous space, inducing oxidative stress [23]. The placenta, however, is armed with protective antioxidant defense enzymes, including the Se-dependent GPx, Trx, selenoprotein P, and copper/zinc and manganese superoxide dismutases [43–45]. Thus, a reduction in selenium concentration will inhibit the Se-dependent antioxidant protection during the early stages of pregnancy.

Conclusion

In this study, we have confirmed our hypothesis that Se may play roles in the development of miscarriage. Antioxidant enzymes, as the primary antioxidants in the human body, are critical for the prevention of miscarriage. An increase in the concentration of free radicals and oxidative stress may be a contributing factor to the occurrence of miscarriage. The present study thus reaffirms that low serum selenium levels are associated with miscarriage and that selenium plays an important role in pregnancy maintenance.

Conflict of Interest None declared

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7