# EFFECTS OF SELENIUM SUPPLEMENTATION ON PLASMA sFlt-1, GPx CONCENTRATION AND PAI1/PAI2 RATIO IN PREGNANT WOMEN

## *Gebe Kadınlarda Selenyum Desteğinin Plazma* sFlt-1, GPx konsantrasyonuna ve PAI1/PAI2 Oranına Etkileri

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### ÖZET

**Amaç:** Preeklampsi gebelikte hipertansif hastalıklardan olup hem maternal, hemde prenatal mortalite ve morbiditeyi artırmaktsdır. Preeklampsi selenyum eksikliğine bağlı olarak yetrsiz glutatyon peroksidaz (GPx) oluşumuna, dolayısıyla antioksidan yetersizliğine bağlı oksidatif stress sonucunda gelişiyor olabilir. Suda eriyebilen fms benzeri tyrosin kinaz 1 (sFlt-1) endotel disfonksiyonuna yol açarak preeklampsinin klinik bulgularından sorumu olabilir. Arıca preeeklamptik kadınlarda plazminogen aktivatör inhibitör -1 (PAI-1) seviyesi yükselirken, plazminogen aktivator -2 (PAI-2) seviyesi düşmektedir. PAI-1/PAI-2 oranı preeklampsi tanısında önemli olabilir. Bu çalışmanın amacı gebekadınlarda verilecek slenyum desteğinin s Flt-1,GPx aktivitesi ve PAI-1/PAI-2 oranı üzerindeki etkilerini araştırmaktır.

**Metod:** Preeklampsi gelişmesi açısından riski yüksek olan 166 kadına (ailesel hipertansiyon öyküsü veya hiperlipidemisi, preeklampsi için diğer risk faktörleri olan) rastgele veya selenyum desteği verilmiştir. Selenyum grubu, altı ay boyunca 100 mikrogram/gün selenyumtableti verilmiştir. PLasebo grubu ise aynı süre ile plesebo tableti kullanmıştır. Termde tüm hastalardan kan örenekleri alınarak serumda sFlt-1,GPx aktivitesi ve PAI-1/PAI-2 aktivitesi çalışılmıştır.

**Sonuçlar:** Birinci trimesterden itibaren Selenyum konsantrasyonu selenyum alan grupta yükselirken ($p<0.05$) plasebo grubunda değişmeden kalmıştır ($p>0.05$). Gebeliğin sonunda her iki grupta da sFlt-1 seviyesinin önemli oranda yükseldiği ($p>0.05$) görüldü. Kontrol grubuyla karşılaştırıldığında selenyum verilen grupta GPx aktivitesindeki artışın daha fazla olduğu ($p<0.05$) saplandı. PAI-1/PAI-2 oranının her iki grupta önemli bir farklılık göstermediği ($p<0.05$) bulundu.

**Yorum:** Gebelikte ikinci ve üçüncü trimesterde selenyum desteği verilmesi sFlt-1 seviyesini yükseltirken, GPx aktivitesini ve PAI-1/PAI-2 oranının değiştirememektedir.

**Anahtar Kelimeler:** FMS benzeri Tyrozin Kinaz-1, Glutatyon peroksidaz, PAI-1/PAI-2 oranı, Preeklampsi, Selenyum

### ABSTRACT

**Objective**: Preeclampsia is a hypertensive disorder of pregnancy, which is associated with increased maternal and prenatal morbidity and mortality. Oxidative stress associated with preeclampsia may be a consequence of reduced antioxidant defense pathways that might involve inadequate glutathione peroxidase (GPx), perhaps linked to reduced selenium availability. The soluble fms-like tyrosine kinase-1 (sFlt-1) contributes to endothelial dysfunction and may be partially responsible for the clinical manifestation of preeclampsia. Furthermore, elevated plasminogen activator inhibitor-1 (PAI-1) and decreased plasminogen activator inhibitor-2 (PAI-2) are found in preeclamptic women. Hence, the PAI1/ PAI2 ratio may be a predictor of preeclampsia. The objective of this study was to evaluate the effects of selenium supplementation on sFlt-1, GPx activity and the PAI1/PAI2 ratio in pregnant women.

**Methods:** A total of 166 high-risk pregnant women (with a familial history of hypertension, hyperlipidemia and other risk factors for preeclampsia) in the first trimesters of pregnancy were selected and randomly allocated to either selenium supplementation or placebo. The selenium group received 100 µg/day selenium as a selenium-yeast tablet for six months. The placebo group received a placebo yeast tablet for the same period. At term, blood samples were collected and the levels of sFlt-1, PAI-1, PAI2 and GPx activity were measured in blood serum.

**Results**: Selenium concentration was increased in blood of the selenium group ($p<0.05$) in the first trimester, but was unchanged in the placebo group ($p >0.05$). The results showed that sFlt-1 had significantly increased in both groups by the end of gestation ($P>0.05$). GPx activity increased in the selenium treatment group after supplementation compared to the control group ($P<0.05$). The PAI1/PAI2 ratio was not significantly different between the two groups ($P>0.05$).

**Conclusion:** Selenium intake in the second and third trimester of pregnancy increased GPx activity concentrations but did not influence the sFlt-1 level nor the ratio of PAI1/PAI2 in the serum.

**Key words:** Fms-like Tyrosine Kinase 1, glutathione peroxidase, PAI1/PAI2 ratio, preeclampsia, selenium

## INTRODUCTİON

Preeclampsia is a significant obstetric problem and is usually characterized by proteinuria, hypertension and edema (1). The prevalence of preeclampsia has been reported to be 2-7% of all pregnancies (2). Although several studies have been performed to understand the etiology of this syndrome, the exact mechanism which leads to preeclampsia is largely unknown (3). Several investigations indicate that oxidative stress in preeclampsia is higher than in normotensive pregnancies (4-6) but even in normal pregnancies it is higher than in non-pregnant women. Hence, prooxidant-antioxidant balance may remain unchanged overall.

Soluble vascular endothelial growth factor (VEGF) receptor-1 is known as an anti-angiogenic protein which acts by inhibition of VEGF and PlGF (placental growth factor), two angiogenic factors which increase during the first two trimesters in preeclamptic women (7). The sFlt-1 levels decline 24 hours post-delivery in both preeclamptic and normal subjects. SFlt-1 is a splice variant form of membrane bound protein that contains an extracellular domain, a trans-membrane domain and an intracellular domain (8). The splice variant mRNA for sFlt-1 has 6 IgG like domains. Excess circulating sFlt-1 in preeclamptic patients causes endothelial dysfunction and leads to an antiangiogenic state. sFlt-1 administration to pregnant rats caused a reduction in blood pressure and VEGF concentrations in comparison with controls. Furthermore, basal superoxide dismutase levels and NADPH production increased in the placenta, renal cortex and rat aorta (9). After placental perfusion is reduced, an increase in PAI-1 and decrease in PAI-2 has been observed in preeclamptic patients. Hence, the ratio of PAI1/ PAI2 may be a useful predictor of preeclampsia (10). The aim of this study was the investigation the effects of selenium supplementation on sFlt-1, GPx level and PAI1/PAI2 ratio in the pregnant women with high risk of preeclampsia.

## MATERİALand METHODS

### Subjects

After approval this study by the Ethical Committee of Mashhad University of Medical Sciences, 218 pregnant women16 to 35 years of age were assessed for eligibility to participate in this trial. These subjects were randomly selected from women referred to the Obstetrics and Gynaecology Department of OM-Albanin and Ghaem Hospital (Mashhad, Iran) between June 2006 and August 2008. The inclusion criteria were gestational age more than 12 weeks with a live fetus and no indication for terminating the pregnancy. Thirty-nine subjects were excluded from the study because of consumption of any drug, except routine supplementations of folic acid and ferrous sulfate, and a history of thyroid dysfunction, diabetes, hypertension or infections.

### Intervention

One hundred and sixty-six remaining subjects were randomly allocated into two groups in a double-blind manner. The selenium group (n=83) received 100 µg/day of selenium, as selenium yeast, for approximately six months, and the placebo group (n=83) used daily placebo yeast tablets for the same period. Thirteen pregnant women could not tolerate the supplements (n=4) or refused to continue

because of the unpleasant aroma associated with tablets (n=9). One hundred and twenty five subjects completed the study (61 cases for selenium group and 64 cases for control group).
Selenium yeast tablets and matching placebo yeast tablets were provided by Pharmanord Vejle (Denmark).

**Sample collection**
Blood samples were taken in the morning from each woman after an overnight fast into plain serum tubes for sFlt-1and selenium measurement and into chilled tubes containing heparin for PAI-1/PAI-2 ratio and GPx measurement. Blood samples were left to clot for 30-60 min and then centrifuged at 2500 rpm for 15 min at room temperature. Serum was stored at -80°C prior to analysis.

**SFlt-1 measurement**
The serum concentration of sFlt-1 was measured using commercial assays kit from R&D systems (Quantikine® Human Soluble VEGF R1/Flt-1 Immunoassay, Catalog number DVR100B). The sensitivity of the assay was 3.5 pg/ml and the intra- and inter-assay variation were 3.2% and 7.4% respectively.

**GPx measurement**
The plasma activity of GPx was measured using Glutathione assay kit (Item No.703102) from Cayman Chemical Company. In this kit, the intra-assay coefficient of variation was 5.7% and inter assay coefficient of variation was 7.2%.

**Measuring of PAI-1 and PAI-2 and calculation of PAI-1/PAI-2**
PAI-1 was measured by ELISA kit[Assay Max Human Plasminogen Activator Inhibitor-1(PAI-1) from AssayPro Company, USA]. The minimum detectable concentration of PAI-1 was 200 pg/ml and the inter- and intra-assay coefficients of variation were 4.9 % and 7.1 %, respectively. The intra- and inter-assay coefficients of variations (CV) for this ELISA kit is 5.6% and 7.8% for high concentration (6ng/ml) and 8.3% and 14.5% for low concentration (1.5 ng/ml) respectively.

**Statistical analysis**
Data were analyzed using SPSS software (version 16). All data were presented as means±SD or median and inter quintile range as appropriate. Comparisons were performed by paired *t*-test for dependent groups and Mann-Whitney U-test for independent groups.

**RESULTS**
The demographic data for the selenium and placebo groups are presented in Table1.
1. Demographic characteristics of selenium and placebo groups at baseline

| Parameters | Selenium group n=61 | Control group n= 64 |
|---|---|---|
| **Age (years)** | 21.6±2.5 | 21.6±3.4 |
| **Weight (kg)** | 58.5±10.2 | 56.5±9.8 |
| **Height (cm)** | 156.7±5.8 | 156.7±6.3 |
| **BMI (kg/m2)** | 23.8±3.8 | 23.0±4.0 |
| **Waist circumference (cm)** | 77.1±11.6 | 4.9±8.2 |
| **Hip circumference (cm)** | 95.8±12.1 | 94.8±7.9 |
| **Waist/hip ratio** | 0.8±0.08 | 0.8±0.06 |
| **SBP (mmHg)** | 100.14 ±10.49 | 101.86±11.41 |
| **DBP (mmHg)** | 61.62 ± 8.2 | 70.16 ± 9.17 |
| **Chol (mg/dl)** | 177.25± 60.16 | 165.88±53.4 |
| **TG (mg/dl)** | 89.18± 34.67 | 88.88±34 |
| **LDL (mg/dl)** | 113.84 ± 54.98 | 104.44±49.85 |

| | | |
|---|---|---|
| **HDL (mg/dl)** | 48.76± 10.37 | 46.77±8.56 |
| **Education Less than 12 years** | 43.9% | 42.2% |
| **Up to 12 years** | 50.5% | 53.3% |
| **More than 12 years** | 5.6% | 4.4% |
| **History of diabetes** | 1.9% | 5.5% |
| **History of hypertension** | 5.6% | 5.5% |
| **History of hyperlipidemia** | 4.7% | 5.5% |

Values are expressed as mean± SD (for normally distributed data) or median and interquartile range (for non- normally distributed data). BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure. Cho: cholesterol. TG: Triglyceride. No Significant difference exists between the groups (p>0.05).

No differences were found in age, anthropometric indices, lipid profile, past medical history (miscarriage and infertility history) or family history (incidence of preeclampsia, diabetes, hypertension and hyperlipidemia) between the two groups.

There was no difference between GPx activity level between the two groups (P=0.08). Selenium supplementation increased plasma GPx level in selenium group (P=0.013) compared to the placebo group (P=0.91).

No significant difference was observed between serum sFlt-1 levels of the two groups at the beginning of the study (P=0.66) but in both groups, sFlt-1 levels were increased at the end of the trial (P<0.05). However, this increase was not significantly different between the two groups (P=0.51). Selenium supplementation had no effect on sFlt-1 level in the selenium group (P=0.781), but in both group sFlt-1 level increase at the end of the trial (P<0.001).

Serum PAI1 and PAI2 concentrations were significantly changed before and after trial (p<0.001). The ratio of PAI1/PAI2 had decreased in case group and its elevated in control groups but these changes were not statistically significant.

**DİSCUSSİON**

Selenium supplementation was found to increase the plasma GPx activity and reduce the PAI1/PAI2 level in the selenium supplemented group compared to the control group, but it had no effect on serum sFlt-1 level.

Increasing the plasma GPx activity in the selenium group while the GPx level in placebo group remains unchanged is a finding that is consistent with our previous study that selenium is reduced during preeclampsia and selenium supplementation increases the serum selenium level in mothers whom consume selenium tablets (11), because selenium can change the pattern of selenoprotein expression.

Furthermore, PAI-1 and PAI-2 levels increased during pregnancy in both the placebo and selenium groups. Chapell and colleagues reported that the ratio of PAI1/PAI2 decrease in pregnant women with low risk of preeclampsia during pregnancy and this ratio increased in pregnant women who developed preeclampsia (10). Other studies (12- 14) have also found a higher PAI1/PAI2 ratio in women with preeclampsia compared to healthy women, and this ratio was proposed as a predictive marker of preeclampsia in these studies, but PAI1/PAI2 ratio is reduced during normal pregnancy. In some studies it was found that PAI1/PAI2 ratio is reduced during low risk pregnancy, while the results of other studies (12, 13), and demonstrate no significant change in this ratio in normal pregnancies. Our data can be interpreted in two ways. The ratio of PAI1/PAI2 in selenium group at beginning of the study was higher than for the placebo group, and was finally less; hence oxidative stress is

decreased in this group. So selenium treatment may have influence on PAI1/PAI2 ratio, a reduced in oxidative stress and as a result reduction in preeclampsia risk.

Many studies have now shown that sFlt-1 may be responsible for the clinical manifestation of preeclampsia. For example, Reddy and colleagues in 2009 reported that serum sFlt-1 levels were higher pre-labour/pre-delivery in preeclamptic pregnancies than in normal pregnancy (15). Furthermore, in pre-eclamptic women, labour increases the levels of sFlt-1 (15).

Another study aimed to investigate the changes of placental growth factor and sFlt-1 are changed during the second trimester in the plasma of 46 women who subsequently develop preeclampsia (16). The PIGF levels were significantly lower in the preeclamptic women than in normal controls, while the sFlt-1 levels were significantly higher. The sFlt-1/PlGF ratio was significantly higher in the preeclamptic women than in the normal controls leading the authors to propose that the sFlt-1/PlGF ratio may be an early predictive marker of subsequent development of preeclampsia (16). These studies are consistent with our finding, but selenium supplementation was not associated with a decrease in sFlt-1 level.

Because arterial changes occur in first trimester, and antioxidants may influence sFlt-1 expression early it maybe that antioxidant supplementation may have an effect if started in the first trimester of pregnancy or even before the gestation. The limitations of our study, include a relatively small sample size and short period of treatment sofurther studies may be required with a larger sample size and starting supplementation from the beginning of the gestation.

Furthermore in our sample population the baseline serum selenium concentrations were not low; so the effect of selenium supplementation may be greater in a region with of lower selenium status. Overall, selenium administration in the second and third trimesters of pregnancy significantly raised the antioxidant level and improved the PAI/1lPAI2 ratio though not to a significant extent. There was no effect on serum sFlt-1 levels though this might be a consequence of the relatively short period of selenium supplementation in this study.

Table 2. Effects of selenium on serum selenium and sFlt-1 levels in the selenium group and control group

| | **Selenium group** | | **Placebo group** | |
|---|---|---|---|---|
| | Pre trial | Post trial | Pre Trail | Post Trail |
| **Selenium** (µg/dl) | 23.25±122.5 | 36.37±168.65* | 26.94±119.41 | 26.90±122.9** |
| **sFlt-1 (**pg/ml) | 1522±518 | 5985±3232* | 1462±462 | 6371±2306* |
| **PAI-1 (**ng/ml) | 54.53±82.94 | 233.37±311.39* | 26.25±29.68 | 138.82±132.26* |
| **PAI-2 (**ng/ml) | 27.83±19.94 | 223.17±195.89* | 42.32±47.65 | 210.46±217.87* |
| **PAI-1/PAI-2** | 1.2(0.23-94.53) | 0.44(0.99-91.1)* | 0.68(0.08-5.24) | 0.96(0.07-91.10)* |

Values are expressed as mean ±SD (for normally distributed data) or median and interquartile range (for non-normally distributed data). Compression between pre- and post-trail values was made using paired t-test for normally distributed data or Wilcoxon test for non- normally distributed data. PAI-1: plasminogen activator inhibitor1. PAI-2: plasminogen activator inhibitor2 sFlt-1: soluble fms like tyrosine kinase. (*P>0.05)(**P<0.001)

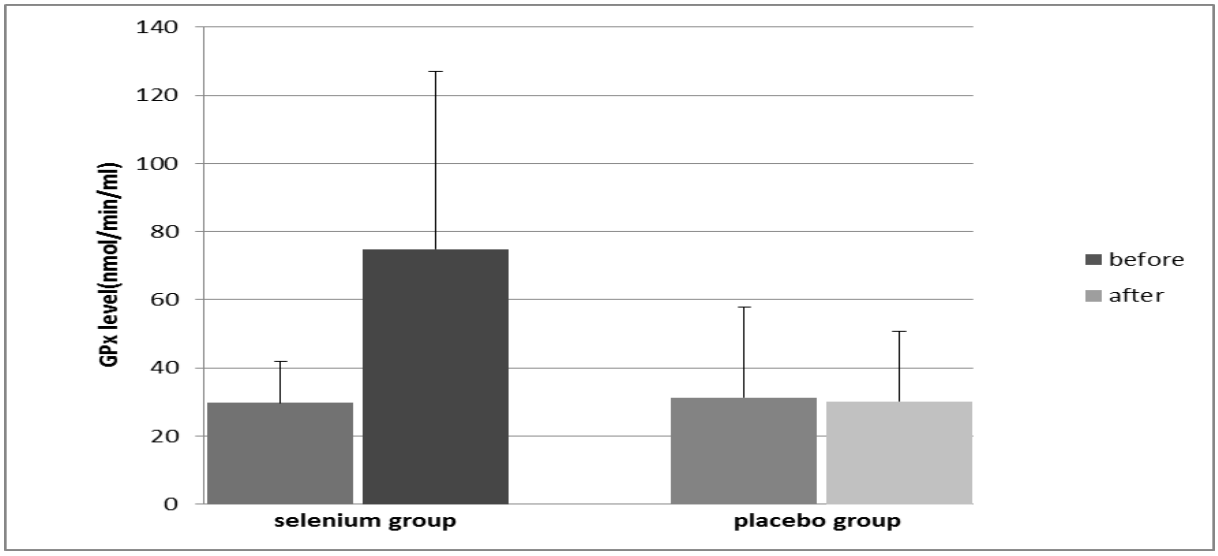


**Figure 1:** Effect of selenium and placebo treatment on plasma GPx activity in pregnant women at high risk of preeclampsia. There was no significant difference in plasma GPx activity between the selenium group and placebo groups before treatment ($P>0.05$). At the end of the trial, there was a significant increase in plasma GPx activity in the selenium group ($p<0.05$) but not in the placebo group.

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