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# Effects of selenium intake on the expression of prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2) and matrix metallopeptidase-9 genes in the coronary artery disease: Selenegene study, a double-blind randomized controlled trial

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Original Article

## Abstract

**BACKGROUND:** The oxidative stress is regarded as one of the main contributors to the health problem. Cyclooxygenase-2 (COX-2) and matrix metallopeptidase-9 (MMP-9) are two of the important genes that are reported to be involved in the cardiovascular disease (CVD) development in the molecular and genetic association studies. The aim of this study was to evaluate the level of expression of COX-2 and MMP-9 after selenium supplementation in patients with coronary artery disease (CAD).

**METHODS:** In this sub-study of Selenegene study, subjects were randomly divided into groups, 19 subjects who received selenium and 22 patients with CAD who received placebo. Patients received either 200-mg selenium yeast tablets or placebo tablets after a meal, once daily for 60 days. The messenger ribonucleic acid (mRNA) levels of the selenium and prostaglandin-endoperoxide synthase 2 (PTGS2) (COX-2) and MMP-9 genes products were determined before and after the study.

**RESULTS:** In this sub-study, 41 Iranian patients with CVD were enrolled (placebo group: n = 22, selenium intervention: n = 19). Fasting blood sugar (FBS) was higher among placebo group than selenium group (93.4 ± 12.7 vs. 124.4 ± 40.6 mg/dl, P = 0.03). Triglyceride (TG) level was higher among selenium group versus placebo group (123.3 ± 34.0 vs. 184.8 ± 69.4 mg/dl, P = 0.006). The data analysis demonstrated that the expression of MMP-9 and COX-2 genes did not change significantly in both selenium and placebo groups.

**CONCLUSION:** This study showed a positive association between the expression of MMP-9 and COX-2 in the patients with CAD who received selenium but not the placebo groups. Yet, these findings need to be confirmed in further details and expanded sample size.

**Keywords:** Selenium; Matrix Metalloproteinase-9; Cyclooxygenase-2; Coronary Artery Disease

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## Introduction

Coronary artery disease (CAD) is a leading cause of mortality, morbidity, and disability in Iranian population. It accounts for nearly 50 percent of all deaths per year. The latest report of the World Health Organization (WHO) has shown that CAD remains one of the leading cause of global mortality.[1,2]

Selenium is a trace element that is critical for many metabolic pathways, including thyroid hormone metabolism, antioxidant defense system, and the immune system function. Several studies have shown that selenium deficiency is linked with

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the certain medical conditions including cancer, cardiovascular disease (CVD), diabetes, and male infertility.[3,4] Studies suggest that selenium may exert beneficial effects on glucose metabolism through many insulin-like actions. Besides, it may prevent diabetes-related vascular disorders.[5] However, the results of several clinical trials and animal models reveal a contradictory effect. To answer that, the recent reports state that there may be a U-shape pattern in response to selenium, that is, the selenium supplementation in the people who have high baseline selenium may increase the risk of diabetes and metabolic disorders. In addition, the beneficial effects of selenium are observed when its concentration is balanced.[6]

The cyclooxygenase-2 (COX-2) enzyme, encoded by the prostaglandin-endoperoxide synthase 2 (PTGS2) gene on the chromosome 1q31.1, is an inflammation-responsive factor that its exact function regarding CAD development needs further researches. The COX-2 generates prostacyclin (PGI2) which has beneficial effect for the cardiovascular health, as it inhibits the leukocytes adherence and migration, platelet aggregation, and the blood clot formation.[7] In addition, the expression of COX-2 is reported to increase the energy expenditure through the activation of the brown adipose tissue (BAT) which leads to weight reduction, improved glucose tolerance, and other metabolic complications.[8] In spite of that, the COX-2 activation is also related with inflammation and insulin-resistance in the adipose tissue.[9,10] Based on some studies, the COX-2 expression is associated with the reactive oxygen species (ROS) production, lower nitric oxide (NO) amounts in the microcirculation, and the subsequent endothelial dysfunction.[11] Moreover, the link between the decreased COX-2 expression and the reduced CAD risk is observed in genetic association studies.[12]

The matrix metalloproteinase (MMP) family are the enzymes in the extracellular environment that are involved in matrix and non-matrix protein degradation. The matrix metallopeptidase-9 (MMP-9) gene is on the chromosome 20q13.12, contains 13 exons and 12 introns, and encodes the MMP-9 or gelatinase-B. The MMP-9 has been shown to have a key role in the atherosclerosis development including vascular remodeling, angiogenesis, and plaque instability. Besides, it is expressed in the endothelial cells, local macrophages, and the peripheral blood mononuclear cells (PBMCs), among the other tissues. In addition, MMP-9 expression and function is reported to be associated with the metabolic syndrome and diabetes complications.[13,14] Moreover, some of the polymorphisms in the MMP-9 gene are reported to be in relevance to CAD risk such as premature CAD development.[15]

Given the prevalence of metabolic syndrome and its important role in predisposing to cardiovascular events, it is imperative to study this condition in more details. Supplementation with selenium may be useful to prevent the progression of metabolic syndrome to CVD. This double-blind clinical trial was designed to determine the differences in the COX-2 and MMP-9 genes expression in cardiovascular patients.

## Materials and Methods

This was a single-center, double-blind, placebo-controlled, randomized clinical trial. All the participants had CAD, which was documented by angiography. In this sub-study, 41 patients were enrolled after fulfilling the inclusion and exclusion criteria. The methodology of Selene gene study has been published elsewhere.[16] The subjects were referred to our research institute from referral heart hospitals in Isfahan, Iran, and the trial was approved by the Research Ethics Committee of Isfahan University of Medical Sciences and conformed to the standards currently applied by the Iranian Registry of Clinical Trial (IRCT = 10252).

*Inclusion criteria:* The age range of 40 to 70 years, lack of any disease including hepatic disorders, kidney disease, gout and arthritis, thyroid and parathyroid disease, adrenal disorders, gynecological diseases, cancer, improved cardiac disease including arrhythmias, uncontrolled congestive heart failure (CHF), severe valvular disease, pericarditis and myocarditis, and lack of CVD and positive familial history for the control group (without a history of CVD).

*Exclusion criteria:* Pregnancy and lactation, hepatic diseases, kidney disease, taking glucocorticoids, hormonal therapy, developing Cushing's syndrome, the risk of inflammatory bowel disease (IBD) and other inflammatory disorders, gastrointestinal (GI) diseases, lactose intolerance, and taking selenium supplements. Patients were asked about taking any vitamins, fiber, omega-3 supplements, or antioxidant during the three weeks prior to the study and if they were positive, they would be considered as confounding.

*Demographic and clinical characteristics of patients:* All volunteering patients were examined for clinical characteristics including age, sex, anthropometric profiles, weight and height, risk of myocardial

infarction (MI), lipid profile, history of heart disease, smoking status, diabetes, high cholesterol background, history of hypertension (HTN), thyroid disease, renal failure, and the patients' medication records. The family history of premature CAD (for men under 55 and women under 65 years), coronary artery bypass grafting (CABG), and percutaneous transluminal coronary angioplasty (PTCA) was also evaluated in the first degree relatives (Table 1). Placebo pills were alike to selenium tablets, and a strategy of numbered boxes was expended for sequence concealment.

*Clinical sampling:* This study was approved by the Ethical Committee of Isfahan University of Medical Sciences. The patients' informed consent was collected. 10 ml venous blood samples were drawn from the patients. Serum samples were obtained from the blood after centrifugation at 1500 g for 10 minutes.

*Biochemical analysis:* The serum cholesterol levels were evaluated using the Roche Hitachi 902 Analyzer (Diamond Diagnostics Inc., Massachusetts, USA) and enzymatic kits (Pars Azmoon Inc., Tehran, Iran). The Friedewald formula was used to calculate the low-density lipoprotein cholesterol (LDL-C) concentrations.

*Ribonucleic acid (RNA) extraction and gene expression analysis:* Total RNA was extracted using RNX™-plus reagent (SinaClon Co., Iran) according to the manufacturer's instructions. Total RNA (3 µg) was treated with deoxyribonuclease I (DNase I) and reverse-transcribed using random hexamers and SuperScript II Reverse Transcriptase (Invitrogen Ltd., UK). Primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and synthesized by Sigma-Aldrich, Ireland. The sequences of primers used for polymerase chain reaction (PCR) are available in table 1. PCR was carried out in a 50 µl mix containing 0.5 µl of Taq polymerase (Invitrogen) and 1 µl of complementary deoxyribonucleic acid (cDNA). PCR products were then run on 2% agarose gel with a parallel 100 bp deoxyribonucleic acid (DNA) ladder (Promega, UK). Real-time PCR was carried out according to the manufacturer's instructions using the LightCycler RNA SYBR Green 1 Amplification Kit (Roche Applied Science, Germany). Data are presented as cycle threshold (Ct). $2^{-\Delta\Delta CT}$ method was performed using the LightCycler software (version 4.0). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression levels were used to normalize. Quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Amplicon), primer pairs (Table 1), and a Corbett Real-Time PCR machine. Gene expression data were normalized against hypoxanthine-guanine phosphoribosyltransferase (HGPRT) as reference gene.[11]

*Statistical tests:* Continues and categorical variables were reported as mean ± standard deviation (SD) and absolute number (percent), respectively. Before all else, the Shapiro-Wilk test of normality was performed. According to test results, student's t-test or Mann–Whitney U test was performed for comparing between two groups based on variable type. In addition, paired t-test or Wilcoxon signed-rank test was used for comparing before and after intervention within groups. Chi-square test was used to compare frequency between categorical variables. SPSS software (version 15, SPSS Inc. Chicago, IL, USA) was used for analyzing. $P < 0.050$ was considered as statistically significant.

## Results

In this sub-study, 41 Iranian patients with CVD were enrolled (placebo group: n = 22, selenium intervention: n = 19). Table 2 displays the demographic characteristics of placebo and selenium intervention groups. No significant differences were observed between either group with regard to age (56.60 ± 8.29 vs. 54.80 ± 7.88 years, P = 0.560), but a significant difference has been found with regards to gender prevalence (women: 13.6% vs. 9.0%, P = 0.021). Fasting blood sugar (FBS) was higher among placebo group than selenium group (93.4 ± 12.7 vs. 124.4 ± 40.6 mg/dl, P = 0.030). Triglyceride (TG) level was higher among selenium group than placebo group (123.3 ± 34.0 vs. 184.8 ± 69.4 mg/dl, P = 0.006). Besides, there were no significant differences with regards to smoking and nutritional habits (e.g., consuming beans, dairy, all types of meats, cereals, nuts, fruits, and vegetables) between the two groups (P > 0.050).

**Table 1.** The prime sequences used for real-time polymerase chain reaction (PCR)

| Gene | Gene expression | Primers | Primer sequence 5'→ 3´ |
|---|---|---|---|
| MMP-9 | NM_004994.2 | Sense | TGGCAGAGGCATACTTGTAC |
| | | Antisense | GTGTTCGAATGGCCTTTAG |
| COX-2 | NM_000963.3 | Sense | TGCAGTGAGCGTCAGGAG |
| | | Antisense | CAAGGATTTGCTGTATGGCTGAG |
| GAPDH | NM_002046.4 | Sense | CCAGTGGACTCCACGACGTA |
| | | Antisense | GCGAGATCCCTCCAAAATCA |

MMP-9: Matrix metallopeptidase-9; COX-2: Cyclooxygenase-2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

**Table 2.** Medical characteristics of the patients

| Demographic characteristics | Placebo (n = 22, 53.67%) | Selenium (n = 19, 46.34%) | P |
|---|---|---|---|
| Age (year) | 56.60 ± 8.29 | 54.80 ± 7.88 | 0.560** |
| SBP (mmHg) | 130.80 ± 17.80 | 126.40 ± 27.90 | 0.620** |
| DBP (mmHg) | 74.90 ± 7.70 | 79.20 ± 16.40 | 0.360** |
| Total cholesterol (mg/dl) | 147.50 ± 20.10 | 168.00 ± 40.90 | 0.100** |
| TG (mg/dl) | 123.30 ± 34.00 | 184.80 ± 69.40 | 0.006$ |
| HDL-C (mg/dl) | 44.30 ± 10.40 | 41.60 ± 11.10 | 0.530** |
| LDL-C (mg/dl) | 78.40 ± 14.10 | 89.40 ± 36.80 | 0.290** |
| FBS (mg/dl) | 93.40 ± 12.70 | 124.40 ± 40.60 | 0.030** |
| Intake of food items | | | |
| Red meat intake (times/week) | 6.82 ± 2.73 | 7.90 ± 5.80 | 0.440$ |
| Fats | 1.32 ± 2.31 | 3.83 ± 3.85 | 0.202$ |
| Fruit and vegetables | 49.60 ± 24.60 | 54.30 ± 21.60 | 0.514$ |
| Nuts | 3.52 ± 3.54 | 3.11 ± 2.77 | 0.690$ |
| Beans | 1.86 ± 0.97 | 2.52 ± 1.38 | 0.087$ |
| Diary | 12.20 ± 4.05 | 12.90 ± 4.65 | 0.603$ |
| Cereals | 22.20 ± 6.81 | 20.50 ± 6.82 | 0.435$ |
| BMI (kg/m$^2$) | 28.40 ± 3.81 | 27.80 ± 4.96 | 0.710** |
| WC (cm) | 98.80 ± 8.61 | 106.80 ± 9.40 | 0.007** |
| Gender (women) | 3 (13.6) | 9 (47.4) | 0.021* |
| Family history of CVD | 1 (4.5) | 4 (21.1) | 0.107* |
| Lifestyle | | | |
| Smoking | 6 (27.3) | 1 (5.9) | 0.840* |

Data are presented as mean ± standard deviation (SD) for continuous variables and absolute number (percent) for categorical variables; * Results from chi-square test; ** Results from independent t-test; $ Results from Mann-Whitney U test
SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; FBS: Fasting blood sugar; BMI: Body mass index; WC: Waist circumference; CVD: Cardiovascular disease

Table 3 shows expression of the genes in both selenium and placebo groups. The data analysis demonstrated that the expression of MMP-9 and COX-2 genes did not change significantly in both selenium and placebo groups.

## Discussion

In the present study, the results show that the expression of MMP-9 is not associated with CAD ($P > 0.050$). The previous investigations have shown the increased pro-MMP-9 plasma levels in the patients with metabolic syndrome. In addition, the upregulated MMP-9 is an indicator of the CAD development in the patients with the hyperlipidemia condition.[14-17]

Although there are some studies on the association between the COX-2 expression and the metabolic syndrome, in this study, the COX-2 expression was not affected in the CAD. In one study, the inhibition of the COX-2 by the celecoxib drug in the fructose-fed rats showed a significant improvement in the CAD features including the systolic blood pressure (SBP) and the insulin-resistance.[18,19] The COX-2 expression is regulated by the inflammatory mediators. Therefore, the higher level of COX-2 expression is observed in the patients with atherosclerosis and in the vascular lesion cells which include the macrophages, endothelial cells, and smooth muscle cells (SMCs).[20] However, the COX-2 inhibitors have been reported to increase the risk of MI in some surveys. In fact, the results of recent studies also declare that there may be a protective effect for the COX-2 against the atherosclerosis development.[21] Moreover, the study by Anwar et al. on the THP-1 cells (monocytes) cells shows that COX-2 inhibition may increase the foam cell formation.[22]

**Table 3.** Analysis of expression of cyclooxygenase-2 (COX-2) and matrix metallopeptidase-9 (MMP-9) genes in the selenium and placebo groups

| Gene | Selenium | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Before | After | P* | Before | After | P* |
| MMP-9 | 4.83 ± 3.00 | 4.34 ± 3.43 | 0.380 | 3.87 ± 2.08 | 10.53 ± 2.37 | 0.120 |
| COX-2 | 8.98 ± 9.21 | 9.21 ± 4.91 | 0.410 | 7.62 ± 0.78 | 10.60 ± 2.91 | 0.890 |

Data are represented as mean ± standard error of the mean (SEM); *Results from Wilcoxon signed-rank test
MMP-9: Matrix metallopeptidase-9; COX-2: Cyclooxygenase-2

The results of the other studies determine that the prostaglandin E2 (PGE2), produced from the arachidonic acid by COX-2, is involved in the regulation of MMP-9 expression from the macrophage cells. Subsequently, the expression of MMP-9 is regulated by the COX-2 function.[22,23] Besides, this relationship between MMP-9 and COX-2 has been also reported in some previous studies and in the cancer cell lines.[24,25]

The current studies emphasize on the possible cardio-protective effects of some minerals including iron, zinc, selenium, and etc., though the reports with respect to the selenium supplements are controversial. Here, we evaluated the possible effects of selenium intake on the expression of MMP-9 and COX-2, the two major genes which their relevance to the CAD has been mentioned earlier. Our results demonstrate that the selenium supplementation does not affect the expression of the selected genes ($P > 0.050$).

The results of a recent study by Liu et al. reveal that the selenium-deficient diet in the mice is accompanied by the increased levels of the COX-2, prostaglandin E (PGE) synthase, and other inflammatory factors.[26] In another study, Hwang et al. reported that the selenate treatment in the colon cancer cells led to the decline in the expression of the COX-2 through the activation of the adenosine monophosphate-activated protein kinase (AMPK) which is a protein kinase involved in the cellular energy equilibrium.[27] Moreover, the study on the murine macrophage cell line (RAW264.7) also showed that in the selenium-insufficient cells, the nuclear factor-kappa B (NF-κB) transcription factor modulated the production of higher levels of COX-2 proteins.[28] In the study by Li et al., the negative effect of selenium supplements on the induction of COX-2 expression in the human umbilical vein endothelial cell (HUVEC) cell line was identified.[29]

There are some preceding studies indicating that the selenium compounds may prevent the cancer invasion by the regulation of MMP-9 gene expression. In one study, the human brain tumor cells were treated with sodium selenite (inorganic form) and the subsequent measurement of gene expression showed a considerable downregulation in the expression of MMP-9.[29] Furthermore, some studies on the other cancer cells including the fibrosarcoma cells and with organic selenium compounds showed a similar result. But, in one study, this was not observed with the selenate compound. For this reason, the distinct effects of each selenium compound should be considered.[30,31]

It should be noted that one of the limitations of this research is the small sample size, since there are restrictions dealing with the human samples. Following studies on the more extended population should be performed. Furthermore, we evaluated the gene expression on the transcription level, and it is necessary to evaluate the changes in the protein levels in the upcoming researches.

Another limitation is related to the gold standard method. To validate the results obtained from the method mentioned in the article, a better option for the authors was to perform present studies on peripheral leukocytes too and evaluate the protein profile with western blot, that is gold standard method, because messenger RNA (mRNA) assay is not an acceptable method to confirm the association between supplements and gene expression.

## Conclusion

The results of this study reveal that the selenium supplementation does not affect the MMP-9 and COX-2 gene expression in the patients with CAD. In addition, the expression pattern of the selected genes is not considerably different in the patients with CAD with respect to the metabolic syndrome. This study also showed a positive association between the expression of MMP-9 and COX-2 in the patients with CAD who received selenium but not the placebo group. Yet, these findings need to be confirmed in further details and expanded sample size.

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## Conflict of Interests

Authors have no conflict of interests.

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