Iran Red Crescent Med J. 2022 May; 24(5):e1563 doi: 10.32592/ircmj.2022.24.5.1563

Published online 2022 May 20 **Original Article**

Effect of Selenium Supplementation on the Levels of Gene Expression Associated with Insulin and Lipid Metabolism, as well as Inflammatory Markers, in Diabetic Hemodialysis Patients

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Received 2021 October 06; **Revised** 2021 December 09; **Accepted** 2022 March 29.

Abstract

Background: One of the major long-term complications of diabetes mellitus (DM) is diabetic nephropathy (DN) characterized by persistent albuminuria and a progressive renal dysfunction.

Objectives: The aim of this trial was to determine the effects of selenium supplementation on the levels of genes expression associated with insulin, lipid and inflammatory markers in diabetic hemodialysis patients.

Methods: This randomized, double-blind, placebo-controlled clinical trial was done on forty diabetic hemodialysis patients. The study subjects were divided into two groups by random to take either 200 µg/day selenium (n=20) or placebo (n=20) during 24 weeks.

Results: Selenium intake led to upregulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) (1.08±0.22 vs. 0.93±0.18 fold change, P=0.049), LDL-receptor (1.06±0.14 vs. 0.90±0.16 fold change, P=0.008) and transforming the growth of factor beta (TGF-β) (1.15±0.18 vs. 0.91±0.20 fold change, P=0.002) in the levels of gene expression. In comparison with placebo, in this intervention also reduction of gene expression of tumor necrosis factor alpha (TNF-α) (0.90±0.19 vs. 1.08±0.19 fold change, P=0.014) and interleukin-1 (IL-1) (1.00+0.11 vs. 1.12+0.15 fold change, P=0.020) were detected. In this study, gene expression of vascular endothelial growth factor (VEGF) (1.02±0.13 vs. 0.96±0.18 fold change, P=0.333) and (IL-8) (0.98±0.20 vs. 1.03±0.17 fold change, P=0.458) were not affected by selenium supplementation.

Conclusion: Selenium supplementation during 24 weeks had positive effects on gene expression associated with metabolic status inflammatory markers in diabetic hemodialysis patients.

Keywords: Diabetic hemodialysis, Inflammation, Gene expression, Metabolic status, Selenium

1. Background

One of the major long-term complications of diabetes mellitus (DM) is diabetic nephropathy (DN) characterized by persistent albuminuria and a progressive renal dysfunction (1). The DN has been indicated to be the main cause of the end-stage renal disease (ESRD) (2). Currently, the number of diabetic patients is going up worldwide; therefore, ESRD is predicted to elevate significantly. Approximately 40% of people with type 2 diabetes mellitus (T2DM) have DN, accounting for 44% of ESRD new cases (3). Almost 95% of these patients are under hemodialysis (HD) treatment [4]. According to recent studies, diabetic hemodialysis patients have a lower 5-year survival rate (5).

Hyperglycemia, the main factor promoting DN, causes abnormal blood flow and glomerulus permeability (6). Moreover, numerous studies indicated that oxidative stress is elevated in DN, leading to the production and accumulation of Advanced Glycation End-products (AGEs) (7). The AGEs and their receptors induce the expression of some cytokines, such as transforming growth factor-β (TGF-β), mediating the transdifferentiation of epithelial cells and formation of myofibroblast leading to tubulointerstitial fibrosis (8). Inflammation is another factor which is seemingly important in DN pathogenesis. Several cytokines, such as TGF-β, vascular endothelial growth factor (VEGF), and profibrotic, have been shown to be increased in DN (9).

Furthermore, the increased expressions and serum levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α), have been found in DN associated with albuminuria (10). The elevated levels of these cytokines promote inflammatory response and cell injury in the kidney, leading to fibrosis (11). Moreover, dysregulated lipid metabolism results in lipids accumulation in the kidney which is important in the pathogenesis of DN. Low-density lipoprotein receptor (LDLR) has a primary function in the regulation of LDL plasma levels (12). Furthermore, PPAR-γ, which is involved in multiple processes, including insulin action, glucose uptake, and lipid metabolism (13), is considerably related to decreased risk of albuminuria in DN. In addition, PPAR-γ has been proposed to play a role in glomeruli (14).

Selenium is known as a pivotal antioxidant and anti-inflammatory micronutrient preventing reactive oxygen species (ROS) production by taking part in the

structure of antioxidant enzymes, such as glutathione peroxidase (GPx) (15). Selenium is found plentifully in organ meats, grain, seafood, dairy, cereals, and nuts (16). A number of studies pointed out that selenium deficiency can lead to oxidative stress which is one of the main processes involved in the progression of diabetes and its complications (17). Recently, the usage of selenium supplementations has been increased due to its probable effects in reducing the risk of some chronic diseases (18). Moreover, some investigations reported that lower concentrations of selenium and GPx in individuals with diabetes mellitus were correlated with vascular complications and microalbuminuria (19). Selenium is involved in the reduction of insulin resistance (IR) index due to its insulin-like function. Furthermore, selenium was found to decrease mitogen-activated protein kinase (MAPK), Nuclear factor-kappa B (NF-κB), and TNF-α levels (20). Moreover, this micronutrient has been observed to elevate the activation of PPAR-γ (21).

2. Objectives

According to these mechanisms, it is hypothesized that selenium administration might have positive effects on diabetic hemodialysis patients. To the best of our knowledge, there is no evidence assessing the impacts of selenium intake on the expression of genes associated with insulin and lipid metabolism, as well as inflammatory markers in diabetic hemodialysis patients. In light of the aforementioned issues, the present clinical trial aimed to investigate the effect of selenium administration on the expression of genes associated with lipid and insulin metabolism, as well as inflammatory markers, in diabetic hemodialysis patients.

3. Methods

The current randomized, double-blind, placebo-controlled, clinical trial study was registered on the Iranian website for clinical trials (http://www.irct.ir: IRCT20170513033941N62). This study included 40 diabetic hemodialysis patients referred to Akhavan Clinic in Kashan, Iran, from July 2019 to February 2020. All participants signed the written consent form. All patients also completed the Declaration of Helsinki requirements. In addition, this study was approved by the Ethics Committee of Islamic Azad University, Yazd Branch. Patients with diabetes mellitus under hemodialysis in the age range of 45-75 years were included in the current study. Furthermore, the exclusion criteria were as follows: having infection, inflammatory conditions, malignancies, administration of selenium or other supplements at least 12 weeks before the beginning of the study, as well as taking such medications as immunosuppressive and antibiotics.

3.1. Sample Size

We used randomized clinical trial sample size calculation formula and regarded PPAR-γ as the primary outcome. Type one (α) was 0.05. Moreover, type two was 0.20 (power=80%). Based on a recent study (22), we regarded 0.15 as the SD, and 0.12 as the mean change (d) of PPAR-γ. The sample size was calculated at 15 cases in each group, and finally, considering 5 dropouts in each group, the sample size increased to 20 patients in each group.

3.2. Randomization

We used random numbers generated by a computer for randomization. The patients and researchers were blinded to the details of randomization until the end of the analysis process. The enrolling, randomization, and assigning of the patients to the groups were performed by dialysis clinic staff.

3.3. Study Design

We asked patients to keep their routine physical activity; moreover, they were asked not to take any antioxidant and anti-inflammatory supplements during the treatment period. Patients gave back us the medication containers to assure that they administered selenium supplements and placebos. Furthermore, the patients were reminded to take the supplement or placebo by sending a short SMS message. The 3-day food records, as well as physical activity records, were obtained from all individuals at weeks 0, 12, and 24. We used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods in order to obtain the macro-and micronutrient intake composition of participants based on their 3-day food records.

3.4. Intervention

In this 24-week intervention, 40 diabetic hemodialysis patients were assigned to two groups: the selenium supplement (200 μg per day) group (n=20) and the placebo group (n=20). Selenium supplements were used as selenium yeast in the current study. Nature MadeCo (California, USA) produced selenium supplement. In addition, placebos were provided by Barij Essence Co (Kashan, Iran). The shape and package of placebo and selenium supplements were similar.

3.5. Assessment of Outcomes

Bodyweight and height were measured in fasting status (by Seca, Hamburg, Germany) at the beginning and the end of the work. Standard formula: [weight (kg)/height (m^2)] was used for the calculation of body mass index (BMI). The primary outcome was the expression of PPAR-γ and the genes associated with lipid metabolism; moreover, inflammatory biomarkers were regarded as secondary outcomes. The extraction of lymphocytes from blood samples was performed to count the cells and perform the

viability test. To extract RNA from cells, an RNX-plus kit was used (Cinnacolon, Tehran, Iran). We frizzed RNA suspension at -20ºC. Extracted RNA was quantified by a UV spectrophotometer. OD 260/280 ratio was contemplated to be in the range of 1.7-2.1, representing no impurity with either DNA or protein.

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to evaluate the gene expressions of PPAR-γ, LDLR, VEGF, TGF-β, IL-1, and IL-8. Light Cycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit was utilized. The housekeeping gene was Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primer Express Software (Applied Biosystems, Foster City, USA) and Beacon designer software (Takaposizt, Tehran, Iran) were used for designing the primers. Relative transcription levels were obtained via the Pffafi method.

3.6. Statistical Analysis

Kolmogorov-Smirnov test was performed for data normality. In addition, the independent-samples t-test detected the differences between both groups in terms of anthropometric factors, dietary consumption, and the expression levels of genes. The effects of treatment on study outcomes were assessed by a multiple linear regression model. P-values less than 0.05 were considered statistically significant. The data were analyzed in SPSS software (version 18).

4. Results

Four subjects in each group were excluded from the study due to personal issues; therefore, 32 patients [selenium (n=16) and placebo (n=16)] finished the trial (Figure 1). In the current research, the participation rate was high. We received no complaints from patients about the side effects of selenium supplementations following the consumption of selenium (Table 1). The two groups had no statistical difference in terms of mean age, height, baseline weight, and BMI (Table 2). According to the 3-day dietary records, no considerable changes were observed in dietary macro- and micro-nutrient intake (Data not shown).

The obtained results pointed out that selenium supplementation elevated gene expression of PPAR-γ (*P=0.049*), TGF-β (*P=0.002*), and LDLR (*P=0.008*) in peripheral blood mononuclear cells (PBMCs) of

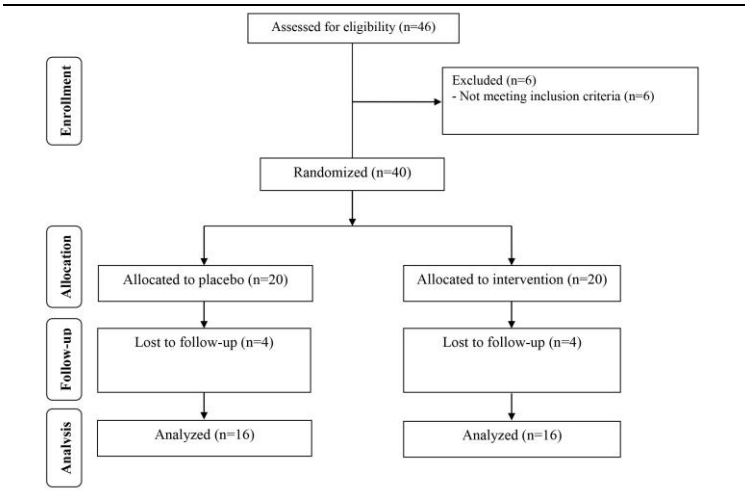

Figure 1. Summary of patients' flow diagram

Table 1. Specific primers used for real-time quantitative polymerase chain reaction

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57
TGF-β	F: TTGAGACTTTTCCGTTGCCG R: CGAGGTCTGGGGAAAAGTCT	227	56
TNF-α	F: GTCAACCTCCTCTCTGCCAT R: CCAAAGTAGACCTGCCCAGA	188	52
IL-1	F: GCTTCTCTCTGGTCCTTGG R: AGGGCAGGGTAGAGAAGAG	174	56
IL-8	F: GCAGAGGGTTGTGGAGAAGT R: ACCCTACAACAGACCCACAC	150	56

GAPDH: glyceraldehyde-3-Phosphate dehydrogenase, PPAR-γ: peroxisome proliferator-activated receptor-gamma, LDLR: low-density lipoprotein receptor, TGF-β: transforming growth factor-beta, TNF-α: tumor necrosis factor-alpha; IL-1, interleukin 1: IL-8, interleukin 8

Table 2. Demographic variables of participants

Variables	Placebo group (n=30)	Intervention group (n=30)	P-value*
Age, year	58.37+9.37	55.00+11.92	0.380
Height, cm	162.81+5.79	168.06+10.68	0.091
Weight, kg	68.28+15.25	74.61+13.36	0.221
BMI, kg/m²	28.27+7.34	26.65+5.37	0.481

Data are means ± SD.
* Obtained from independent t-test.

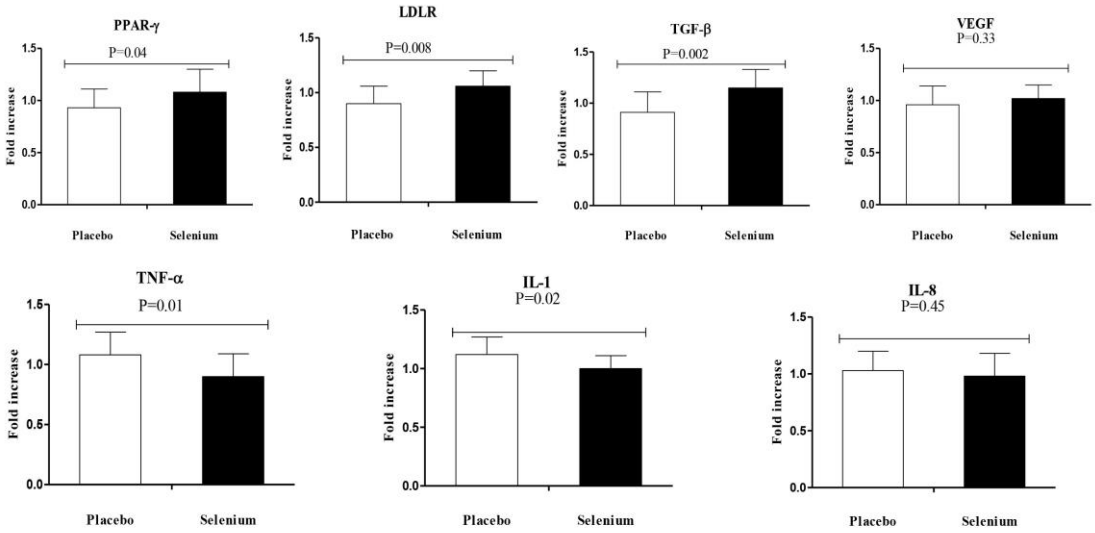

Figure 2. Effect of the 24-week supplementation with selenium or placebo on expression ratio of PPAR-γ, LDLR, TGF-β, TNF-α, VEGF, IL-1, and IL-8 gene in PBMCs of patients with diabetes on HD, Fold changes are displayed as mean±SD.
LDLR: low-density lipoprotein receptor, HD: hemodialysis, PBMCs: peripheral blood mononuclear cells, PPAR-γ: peroxisome proliferator-activated receptor gamma, TGF-β: transforming growth factor-beta, TNF-α: tumor necrosis factor-alpha, VEGF: vascular endothelial growth factor, IL-1:interleukin-1, IL-8: interleukin-8

subjects. Moreover, selenium intake significantly decreased the gene expression of TNF-α (P=0.014) and IL-1 (P=0.020); however, selenium administration did not exert any impact on gene expressions of IL-8 and VEGF (Figure 2).

5. Discussion

The present study aimed to analyze the effect of selenium intake on the expressions of genes associated with insulin and lipid metabolisms, as well as inflammatory markers in diabetic hemodialysis patients. Based on the obtained results, selenium intake elevated the gene expressions of PPAR-γ and LDLR in diabetic hemodialysis patients. Although many studies reported the selenium impact on other metabolic diseases, such as T2DM, obesity, and cardiovascular patients, no study exists assessing the role of selenium intake in metabolic and inflammatory markers in diabetic hemodialysis patients. A meta-analysis study pointed out that selenium intake was useful for attenuating lipid profiles (23). In another study, the administration of 200 µg/day of selenium for 12 weeks reduced plasma levels of insulin and insulin resistance in patients with DN (24).

In line with the results of the current study, an investigation reported that 200 µg/day of selenium increased the gene expression of PPAR-γ in women with polycystic ovarian syndrome for 8 weeks (25). In another study, selenium intake with the same dose led to upregulated gene expression of PPAR-γ, whereas it did not have any impact on the expression of the LDLR gene (26). Moreover, an *in vivo* study reported that selenium (Na2SeO4, 20 µmol/L) increased PPAR-γ expression in 16 weeks (27). In a similar vein, another *in vivo* study declared that selenium (1 ppm) led to increased expression of LDLR (28).

The PPAR-γ is a potential regulator for lipids and carbohydrates metabolism, resulting in the elevation of glucose uptake and fatty acids oxidation (29). Although the exact mechanism in which selenium upregulates PPAR-γ is not fully understood, it is proposed that selenium may regulate p38 mitogen-activated protein kinase (p38 MAPK), elevating the gene expression of PPAR-γ and improving glucose metabolism and insulin resistance (30,31). In addition, selenium promotes insulin metabolism by decreasing ROS formation and regulating other processes, such as phosphatidylinositol 3-kinase (PI3K) and c-Jun N-terminal/ stress-activated kinase (31). On the other hand, sterol regulatory element-

binding protein 2 regulates the gene expression of LDLR which is involved in reducing the plasma levels of LDL cholesterol. Based on investigations, selenium might affect the function of this translation factor by improving the function of thyroid hormones (32,33).

Based on the results, selenium supplementation significantly reduced the gene expressions of TNF-α and IL-1 and elevated the gene expression of TGF-β. Although selenium could not change the expression of VEGF and IL-8 genes, several studies evaluated the role of selenium in inflammatory and oxidative stress processes by evaluating their markers in various metabolic disorders. Recently, a meta-analysis study demonstrated that selenium intake improved inflammation and oxidative stress in patients suffering from cardiovascular disease (34).

In addition, a three-month administration of selenium (200 µg/day) in hemodialysis patients increased GPx function (35). Jamilian et al. (36) reported that selenium supplementation (200 µg/day) elevated VEGF gene expression and reduced TNF-α and TGF-β gene expression in six weeks. Nonetheless, it could not change the expression of the IL-8 gene `in women with gestational diabetes. Another study indicated that a daily intake of 200 µg of selenium upregulated VEGF expression and downregulated TNF-α and IL-1; however, it did not change IL-8 and TGF-β expressions (37). An animal study demonstrated that selenium decreased gene expressions of TNF-α and IL-1 in hepatic cells (38). Inflammation is an effective factor involved in vascular dysfunction, atherosclerosis, and mortality in diabetic hemodialysis patients (39).

Studies have illustrated that upregulated nuclear factor-kappa beta (NF-kB) in selenium deficiency resulted in decreased expression of TGF-β and increased expression of TNF-α and IL-1, leading to inflammation. Therefore, it is suggested that selenium increases TGF-β expression and decreases TNF-α and IL-1 expression by inhibiting the NF-kB pathway (40). The discrepancy in the results of different studies can be ascribed to several factors, including the type of the study, selenium dosage, and intervention duration. Among the notable limitations of this study, we can refer to budget restrictions, inability to determine the effects of selenium administration on other biomarkers of oxidative stress and inflammation, non-use of a biomarker to check compliance to selenium intake, inability to measure variables in the protein levels, and non-evaluation of serum levels of selenium before the intervention.

6. Conclusion

As evidenced by the results of the current study, selenium supplementation for 24 weeks could beneficially affect the gene expressions of PPAR-γ and LDLR, as well as inflammatory markers, including IL-1, and IL-8; nonetheless, it could not significantly affect the gene expressions of TGF-β and VEGF in diabetic hemodialysis patients. Accordingly, further longer-term studies are recommended to be conducted on larger sample size. Moreover, the assessment of the gene expression and protein levels of other related biomarkers, such as kidney-associated factors, can be considered and enhance the current results.

Acknowledgments

The present study was supported by the Islamic Azad University-Yazd branch, Yazd, Iran. The authors would like to thank the staff of Akhavan Clinic (Kashan, Iran) for their assistance in this project.

Footnotes

Conflicts of Interest: The authors declare that they have no conflict of interest.

Author's Contributions: MS contributed to conception, design, statistical analysis, and manuscript drafting. AJ and MD contributed to data collection and manuscript drafting. All authors approved the paper for submission.

Funding Resources: The study was supported by a grant from Islamic Azad University, Ashkezar, Yazd, Iran.

Ethical Statements: This study was approved by the Ethics committee of the Islamic Azad University-Yazd Branch. (Ethics code: IR.IAU.YAZD.REC.1398.022)

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