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Research Article

The Effect of Ascorbic Acid and Calcitriol on the Expression of Vascular Cell Adhesion Molecule-1 (V-CAM 1) in Aortic Early Atherosclerosis: An *In Vivo* Study in a Mouse Model of Atherosclerosis

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Abstract

Background: VCAM-1 expression increases under conditions of high bioactive lipids such as dyslipidemia through the activity of oxidised LDL and triggers the early process of atherosclerosis. Ascorbic acid and Vitamin D are thought to have beneficial roles in aspects of suppressing inflammation, preventing oxidative stress and lowering lipid profiles in subjects at risk of cardiovascular disease.

Method: This research is pure experimental research. The research design used was a posttest design with a control group (post-test only control group design). The research will be conducted at the Animal Experimental Unit and Anatomy Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. The samples of this study were 24 male *Rattus novergicus* strain Wistar white rats obtained from the Bogor Agricultural University (IPB) which were divided into control group (N), Dyslipidemia group (DL) given atherogenic feed, dyslipidemia group given atherogenic feed and vitamin C (DLC) and dyslipidemia group given atherogenic feed and Calcitriol (DLD). Mice will be given ascorbic acid at a dose of 500 mg/day and calcitriol 0.25 mcg/day. Immunohistochemical (IHK) examination of VCAM-1 expression was performed by Kiernan's method with modifications, and immunohistochemical staining method performed is the ABC method. Data on the distribution of VCAM-1 in the cardiac aortic tissue of white rats were analysed descriptively and presented in the form of histological images and analysed using the Kuskal-Wallis non-parametric test and any differences in VCAM-1 expression in the observed sections between treatment groups were analysed by the Mann-Whitney U test.

Results: This research had 24 samples consisting of 6 samples of control group (N), 6 samples of dyslipidemia group (DL) given atherogenic feed, 6 samples of atherogenic feed and vitamin C (DLC), 6 samples of atherogenic feed and Calcitriol (DLD). The results of this study showed that there was a significant difference in VCAM-1 expression between the dyslipidemia group (DL) given atherogenic feed and samples of atherogenic feed and vitamin C (DLC) (p=0.002). Likewise, the atherogenic feed and Calcitriol (DLD) group which was compared with the dyslipidemia group (DL) given atherogenic feed, was found to have a significant difference (p=0.002). However, comparison of VCAM-1 expression between atherogenic feed and vitamin C (DLC) and atherogenic feed and Calcitriol (DLD) groups was found to have no significant difference (p=0.394).

Conclusion: The administration of vitamin C and calcitriol has a positive effect and benefit in reducing VCAM-1 expression *in vivo* study a mouse model of atherosclerosis.

Keywords: Vitamin C; Calcitriol; VCAM-1 expression; Atherosclerosis

Introduction

Atherosclerosis occurs due to lipid accumulation in the arterial wall and sclerosis due to increased lipid accumulation, inflammation, oxidative stress/oxidation of lipids and proteins [1-4]. Low shear stress or haemodynamic forces will create injury to the arterial endothelium. Arterial endothelial cells will dysfunction and respond by releasing inflammatory mediators. Endothelial injury will also be exacerbated by hyperlipidaemia which triggers Low Density Lipoprotein (LDL) oxidation, this process will trigger free radicals and chronic inflammation which will be accompanied by the release of adhesion molecules and accumulation of macrophages migrating to the endothelial layer to form fat foam cells, atheroma and atherosclerotic plaque [3,4].

Various adhesion molecules, including Vascular Cell Adhesion Molecule-1 (VCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1), E-selectin, and P-selectin are expressed on the endothelial surface, with recruitment of monocytes and T cells along with other mediators and platelets. Vascular Cell Adhesion Molecule-1 or cluster of differential 106 (CD106) is a type 1 transmembrane protein expressed on the endothelium. Once VCAM-1 is expressed on the surface of endothelial cells, it will interact with integrin 1β4 present on the surface of circulating leukocytes, allowing coiling, trapping and activation of leukocyte intracellular

signalling. This leads to disruption of endothelial intercellular links and actin remodelling, facilitating the transmigration of monocytes into the arterial wall [4,5].

VCAM-1 expression increases in conditions of high bioactive lipids such as dyslipidemia through the activity of oxidised LDL and triggers the initial process of atherosclerosis. There are several research reports that prove the association of VCAM-1 with the occurrence of atherosclerosis. Aortic VCAM-1 expression is associated with the severity of atherosclerosis and cardiovascular risk factors [6]. In relation to the incidence of myocardial infarction, VCAM-1 expression was found to be 73% higher compared to patients with stable CAD. VCAM-1 density was also significantly higher and positively correlated with the degree of vascular inflammation in patients with myocardial infarction [7].

Ascorbic acid is thought to have beneficial roles in the aspects of suppressing inflammation, preventing oxidative stress and lowering lipid profiles in subjects with cardiovascular disease risk. *In vitro* or theoretically ascorbic acid is reported to inhibit atherogenesis in blood vessel wall cells by inhibiting LDL oxidation and further taking up ox-LDL [8-10]. Besides ascorbic acid, another antioxidant that is also able to suppress inflammation in atherogenesis and dyslipidemia is calcitriol or vitamin D. This vitamin is reported *in vitro* to have the potential to reduce the expression of pro-inflammatory cytokines TNFα, IL-6, IL-1, and IL-8. The suppression of these cytokines leads to a decrease in inflammatory C Reactive Protein (CRP) associated with atherosclerosis. Calcitriol deficiency has also been reported to be associated with dyslipidaemia, where there is an inverse correlation between calcitriol and LDL cholesterol and triglyceride levels, and a positive correlation with HDL cholesterol levels [11-12]. However, many clinical trial studies examining the effectiveness of these 2 vitamins have failed to prove their effectiveness. This study aimed to determine the effects of ascorbic acid and calcitriol on endothelial dysfunction due to oxidative stress by reducing the expression of VCAM-1 in the aorta in a rat model of atherosclerosis.

Methods

This research is a pure experimental research. The research design used was a posttest design with a control group (posttest only control group design). The research will be conducted at the Animal Experimental Unit and Anatomy Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. The samples of this study were 24 male *Rattus novergicus* strain Wistar white rats obtained from the Bogor Agricultural University (IPB) aged 4 weeks, healthy with a body weight of 50 grams-100 grams, which were divided into control group (N), dyslipidemia group (DL) given atherogenic feed, dyslipidemia group given atherogenic feed and vitamin C (DLC) and dyslipidemia group given atherogenic feed and Calcitriol (DLD). Mice will be given ascorbic acid at a dose of 500 mg/day and calcitriol 0.25 mcg/day. Immunohistochemical (IHK) examination of VCAM-1 expression was performed by Kiernan's method with modifications, and immunohistochemical staining method performed is the ABC method. Data on the distribution of VCAM-1 in the cardiac aortic tissue of white rats were analysed descriptively and presented in the form of histological images and analysed using the Kuskal-Wallis non-parametric test and any differences in VCAM-1 expression in the observed sections between treatment groups were analysed by the Mann-Whitney U test (Figures 1-4).

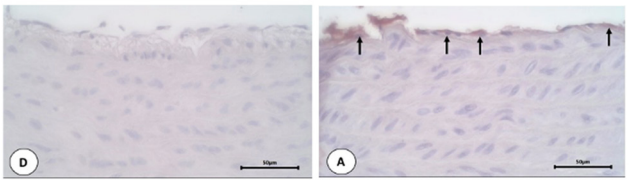

Figure 1: Staining distribution of VCAM-1 expression in cardiac aortic tissue of male Wistar strain white rats (*Rattus novergicus*) in healthy group (K-) and atherosclerosis group (A). Observation of preparations using a microscope with 400x magnification and image analysis using ImageJ 1.53c software

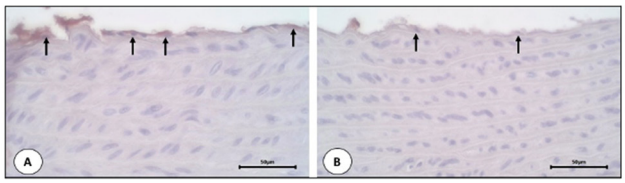

Figure 2: Staining distribution of VCAM-1 expression in cardiac aortic tissue of male Wistar strain white rats (*Rattus novergicus*) in atherosclerosis group (A) and atherosclerosis group with ascorbic acid intervention (B). Observation of preparations using a microscope with 400x magnification and image analysis using ImageJ 1.53c software

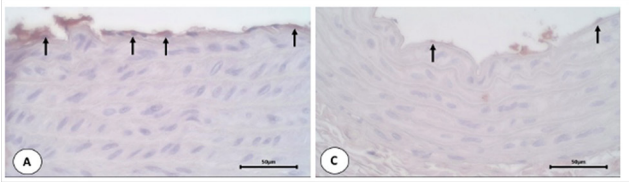

Figure 3: Staining distribution of VCAM-1 expression in cardiac aortic tissue of male Wistar strain white rats (*Rattus novergicus*) in atherosclerosis group (A) and atherosclerosis group with calcitriol intervention (C). Observation of preparations using a microscope with 400x magnification and image analysis using ImageJ 1.53c software

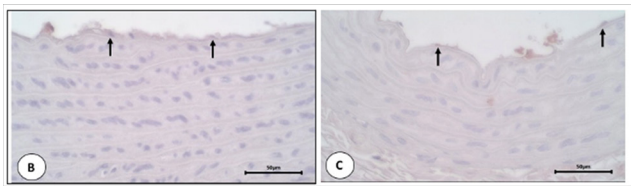

Figure 4: Staining distribution of VCAM-1 expression in cardiac aortic tissue of male Wistar strain white rats (*Rattus novergicus*) in atherosclerosis group with ascorbic acid intervention (B) and atherosclerosis group with calcitriol intervention (C). Observation of preparations using a microscope with 400x magnification and image analysis using ImageJ 1.53c software

Experimental animal care

The welfare of experimental animals is one of the priorities during the research period. Animal welfare is the condition in which experimental animals, which are kept specifically for experimental purposes, have appropriate physiological and psychological conditions to support their quality of

life. Supervision of the implementation of animal welfare in this study involves a veterinarian who will evaluate the condition of the experimental animals twice a week. Cages were made with an area of 200 cm^2 with a height of 20 cm with husk bedding. The cage was cleaned with 25% ethanol solution once a week. The chaff was replaced approximately twice a week. Drinking water is available without restriction and can be given in a bottle with a pipe fitted with a round bullet valve located at the end of the pipe. To prevent the growth of germs, drinking water can be acidified or chlorised.

Atherogenic diet

The diet was implemented by feeding a diet consisting of Vitamin D3, 0.2% cholic acid, 2% egg yolk, 5% goat fat, and 92.8% standard feed. The feed was prepared by mixing 1000 grams of corn rice, 100 grams of goat fat, and 50 grams of egg yolk. Goat fat was preheated to melt, egg yolk was obtained from boiled eggs. The goat fat and egg yolk were then mixed into 1000 grams of maize rice. Feed is given at 10% of body weight per day.

Clinical protocol and participant

The study began with the preparation stage of the experimental animals. The preparation phase aims to acclimatise the rats to the new environment for 2 weeks. During this phase, all rats were fed a normal diet. A normal diet is a diet with the usual composition of animal feed (without the addition of ingredients that can increase cholesterol levels). At the beginning of week 3, 6 rats were kept on the normal diet while 18 rats were randomly selected for the DL rat model. The amount of rat feed consumed will be calculated daily by weighing the remaining feed in the rat cage. How to calculate feed intake is the feed that was given yesterday minus the remaining feed. Body weight measurements are taken every week to determine the weight gain of each rat. LDL examination was carried out in the 9th week after giving atherogenic feed for 8 weeks. In a previous study on the duration of atherogenic feeding on the lipid profile of male Wistar strain white rats, it was found that atherogenic feeding for 8 weeks in rats could significantly increase blood cholesterol levels and induce the formation of foam cells.

In this phase, the experimental animals were treated with ascorbic acid at a dose of 9 mg/day and Calcitriol 0.009 mcg/day *via* oral sonde. While the control group received regular feed diet. The treatment of ascorbic acid and calcitriol was carried out for 30 days. In the final stage, euthanasia was performed by injecting ketamine 15 mg/kg-20 mg/kg per intra peritoneal and performed with cervical dislocation. Anaesthesia and surgery were performed by experienced researchers and laboratory staff. The heart aorta of the experimental animals was taken and cut and fixed in 10% neutral buffered formalin solution for 48 hours. After fixation, the samples were transferred into 70% alcohol solution as a stopping point.

Statistical analysis

Data on the distribution of VCAM-1 in white rat heart aorta tissue were analysed descriptively and presented in the form of histology images. IS data on VCAM-1 expression were analysed using the Kuskal-Wallis non-parametric test and any differences in VCAM-1 expression in the observed sections between treatment groups were analysed using the Mann-Whitney U test (Figure 5).

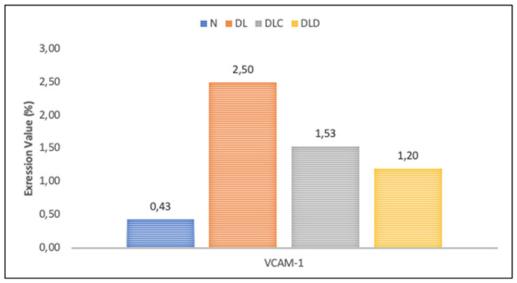

Figure 5: Comparison of VCAM-1 expression in aortic tissue of white rat heart in healthy group, atherosclerosis group (DL), atherosclerosis group with calcitriol (DLD) and ascorbic acid (DLC) intervention

Result

This research is pure experimental research. The research design used was a posttest design with a control group (posttest only control group design). The purpose is to effects of ascorbic acid and calcitriol administration in reducing VCAM-1 expression in the aorta in a rat model of atherosclerosis. This research was carried out in August until November 2022.

Expression of VCAM-1 in control and atherogenic feed groups (DL)

Table 1 shows that the VCAM-1 expression in the group of healthy rats has a significant difference with the group given atherogenic feed (LD) with a p value of 0.002. In addition, the value of VCAM-1 expression in the group of rats that experienced atherosclerosis increased, where in the LD group the VCAM expression was 2.57 ± 0.29, while the healthy rat group was 0.43 ± 0.15.

Table 1: Expression of VCAM-1 in control and atherogenic feed groups (DL)

VCAM-1	N	DL	P value
Expression activity (distribution percentage), %	0.43 ± 0.15	2.57 ± 0.29	0.002

Expression of VCAM-1 in atherogenic feed groups (DL) and atherogenic feed and ascorbic acid (DLC)

Table 2 shows that the VCAM-1 expression in the group of atherogenic feed groups (DL) has a significant difference with the group given atherogenic feed and ascorbic acid (DLC) with a p value of 0.002. In addition, the value of VCAM expression in the group of rats that experienced atherosclerosis with vitamin C administration decreased, where in the DLC group the vcam expression was 1.53 ± 0.30, while the DL group was 2.57 ± 0.29.

Table 2: Expression of VCAM-1 in atherogenic feed groups (DL) and Atherogenic feed and ascorbic acid (DLC)

VCAM-1	DL	DLC	P value
Expression activity (distribution percentage), %	2.57 ± 0.29	1.53 ± 0.30	0.002

Expression of VCAM-1 in atherogenic feed groups (DL) and atherogenic feed and calcitriol (DLD)

Table 3 shows that the VCAM-1 expression in the group of atherogenic feed groups (DL) has a significant difference with the group given Atherogenic feed and calcitriol (DLD) with a p value of 0.002. In addition, the value of VCAM expression in the group of rats that experienced atherosclerosis with calcitriol administration decreased, where in the DLD group the vcam expression was 1.20 ± 0.55, while the DL group was 2.57 ± 0.29.

Table 3: Expression of VCAM-1 in atherogenic feed groups (DL) and atherogenic feed and calcitriol (DLD)

VCAM-1	DL	DLD	P value
Expression activity (distribution percentage), %	2.57 ± 0.29	1.20 ± 0.55	0.001

Expression of VCAM-1 in Atherogenic feed and ascorbic acid (DLC) and atherogenic feed and calcitriol (DLD)

Table 4 shows that the expression of VCAM-1 in the group fed with atherogenic feed and ascorbic acid (DLC) did not have a significant difference with the group fed with atherogenic feed and calcitriol (DLD) with a p value of 0.394.

Table 4: Expression of VCAM-1 in Atherogenic feed and ascorbic acid (DLC) and atherogenic feed and calcitriol (DLD)

VCAM-1	DLC	DLD	P value
Expression activity (distribution percentage), %	1.20 ± 0.55	1.53 ± 0.30	0.394

Discussion

This research aims to effects of ascorbic acid and calcitriol administration in reducing VCAM-1 expression in the aorta in a rat model of atherosclerosis. The samples of this study were 24 male *Rattus novergicus* strain Wistar white rats obtained from the Bogor Agricultural University (IPB) aged 4 weeks, healthy with a body weight of 50 grams-100 grams, which were divided into control group (N), dyslipidemia group (DL) given atherogenic feed, dyslipidemia group given atherogenic feed and vitamin C (DLC) and dyslipidemia group given atherogenic feed and calcitriol (DLD).

The findings show that the administration of vitamin C and calcitriol can decrease VCAM-1 expression in the aorta. There are several research reports on the role of ascorbic acid in lipid metabolism and atherogenesis. It has been reported to aid in the reduction of triglyceride cholesterol, per-oxidised lipids and increase HDL cholesterol. Ascorbic acid inhibits the oxidation of LDL proteins, thereby reducing atherosclerosis, but the results of cardiovascular field research related to the therapeutic actions with this vitamin C are not fully understood. Randomised controlled trials and observational cohort studies have investigated this vitamin with varying results, attributing some improvements in lipid profiles, improvements in arterial stiffness and endothelial function [13]. In a metaanalysis report on ascorbic acid supplementation, it was reported to improve endothelial function and appeared to be dependent on health status. The benefits of ascorbic acid were found to be stronger in those with cardio-metabolic disorders and ascorbic acid doses greater than 500 mg/day were associated with beneficial effects on endothelial function [14].

The antioxidant effect of ascorbic acid on inhibiting atherogenesis in vascular wall cells is thought to be through reducing cellular production and release of Reactive Oxygen Species (ROS), inhibiting endothelial activation (i.e., expression of adhesion molecules and monocyte chemoattractants), and increasing the biological activity of endothelial-derived nitric oxide. Regarding adhesion molecules triggered by oxidative stress, a significant association between increased SOD levels, decreased VCAM-1 levels in imminent abortion after ascorbic acid administration was reported. In another research report related to atherosclerosis conditions, administration of ascorbic acid and vitamin E in smokers with hyperaemic conditions showed a decrease in atherosclerosis-related adhesion molecules. Short-term administration of ascorbic acid (2 g/day) and E (800 IU/day) decreased serum levels of IL-1b, IL-6, sVCAM-1 and ICAM-1, and improved forearm vasodilatory response to reactive hyperaemia in healthy people and young smokers [8,15]. The inflammatory response seen in atherosclerosis may be inhibited by ascorbic acid by preventing leucocyte aggregation and adhesion to endothelium, induced by cigarette smoke *in vitro*. Ascorbic acid also has the potential to prevent lipid peroxidation, the formation of atherosclerosis, by inhibiting LDL oxidation and subsequently taking up ox-LDL [8-10].

Calcitriol is recognised as an essential micronutrient for calcium and phosphorus metabolism, which are important for muscle and bone tissue. However, calcitriol may also influence the pathophysiology of cardiovascular disease through several mechanisms. There are several supporting research reports including calcitriol can reduce lipid profile, suppress endothelial adhesion, increase nitric oxide and prevent free radicals, suppress proinflammatory cytokines, reduce oxidative stress production and reduce angiotensin production [8,9].

There are reports of 25(OH)D level being shown to be one of the significant determinants of CAD. In subjects without significant lesions in the coronary arteries, 25(OH)D levels were significantly higher compared to patients with 1-3 vessel coronary atherosclerosis ($p<0.05$). Significantly higher 25(OH)D levels were recorded in patients diagnosed with stable CAD compared to patients hospitalised for acute coronary syndrome ($p<0.01$) [16,17].

Calcitriol can reduce the expression of TNFα, IL-6, IL-1, and IL-8 in isolated blood monocytes. Suppression of IL-6 leads to a decrease in inflammatory C Reactive Protein (CRP). The serum concentration of CRP is associated with atherosclerosis and serves as a predictor of cardiovascular events [18-21]. Calcitriol deficiency was shown to accelerate the progression of coronary artery disease in experimental animals by increasing nuclear factor-κB (NF-κB) activation, indirectly supporting the anti-inflammatory role of calcitriol. Macrophage-derived foam cells

characterise the development of early atherosclerosis. Calcitriol was shown to reduce cholesterol accumulation in macrophages and LDL uptake in atheroma. In addition, it modulates thrombomodulin and tissue factor expression in monocytes, affecting platelet aggregation and thrombogenic activity. Moreover, calcitriol 1,25$(OH)_2$D reduces the expression of matrix metalloproteinase (MMP)-2 and MMP-9 in cell culture, thus possibly preventing plaque destabilisation, luminal rupture, and thrombosis [17].

Conclusion

The administration of vitamin C and calcitriol has a positive effect and benefit in reducing VCAM-1 expression *in vivo* study a mouse model of atherosclerosis.

Ethics Approval and Informed Consent

The research approval was obtained from the health research ethics commission, Faculty of Veterinary Medicine, Syiah Kuala University based on the issuance of a Certificate of Passing Ethical Review (160/KEPH/VIII/2022).

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding

None.

Authors' Contributions

All authors significantly contribute to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas. Contribute to drafting, revising, or critically reviewing the article. Approved the final version to be published, agreed on the journal to be submitted, and agreed to be accountable for all aspects of the work.

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Conflict of Interest

Authors have no conflict of interest to declare.

References

1. World Health Organization, Cardiovascular Diseases (CVDs), 2021.
2. Hasil riset kesehatan dasar tahun, Kementrian Kesehatan, Indonesia, 2018.
3. L.S. Lilly, Pathophysiology of heart disease: A collaborative project of medical students and faculty, 2016.
4. M.F. Troncoso, J. Ortiz-Quintero, V. Garrido-Moreno, F. Sanhueza-Olivares, A. Guerrero-Moncayo, et al. VCAM-1 as a predictor biomarker in cardiovascular disease. Biochimica et Biophysica Acta (BBA)-molecular basis of disease, Biochim Biophys Acta Mol Basis Dis, 1867(2021):166170.
5. M. Yin, C. Li, J. Le, B. Luo, F. Yang, et al. Cell adhesion molecule-mediated therapeutic strategies in atherosclerosis: From a biological basis and molecular mechanism to drug delivery nanosystems, Biochem Pharmacol, 186(2021):114471.
6. W. Mu, M. Chen, Z. Gong, F. Zheng, Q. Xing, Expression of vascular cell adhesion molecule-1 in the aortic tissues of atherosclerotic patients and the associated clinical implications, Exp Ther Med, 10(2015):423-428.
7. C.E. Radecke, A.E. Warrick, G.D. Singh, J.H. Rogers, S.I. Simon, et al. Coronary artery endothelial cells and microparticles increase expression of VCAM-1 in myocardial infarction, Thromb Haemost, 113(2015):605-16.
8. B. Frei, On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction, Proc Soc Exp Biol Med, 222(1999):196-204.
9. B. Frei, Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage, Am J Clin Nutr, 54(1991):1113S-1118S.
10. H.A. Lehr, B. Frei, K.E. Arfors, Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium *in vivo*, Proc Natl Acad Sci, 91(1994):7688-92.
11. R.C.M. Siow, J.P. Richards, K.C. Pedley, D.S. Leake, G.E. Mann, Vitamin C protects human vascular smooth muscle cells against apoptosis induced by moderately oxidized LDL containing high levels of lipid hydroperoxides, Arterioscler Thromb Vasc Biol, 19(1999):2387-94.
12. T. Gong, H. Di, X. Han, X. Hu, C. Liu, et al. Vitamin D is negatively associated with triglyceride in overweight/obese patients with type 2 diabetes, Endocrine, 76(2001):304-311.
13. M.A. Moser, O.K. Chun, Vitamin C and heart health: A review based on findings from epidemiologic studies, Int J Mol Sci, 17(2016):1328.
14. A.W. Ashor, J. Lara, J.C. Mathers, M. Siervo, Effect of vitamin C on endothelial function in health and disease: A systematic review and meta-analysis of randomised controlled trials, Atherosclerosis, 235(2014):9-20.
15. D. Tousoulis, C. Antoniades, C. Tentolouris, C. Tsioufis, M. Toutouza, et al. Effects of combined administration of vitamins C and E on reactive hyperemia and inflammatory process in chronic smokers, Atherosclerosis, 170(2003):261-7.
16. E.A. Dziedzic, J.S. Gąsior, M. Pawłowski, B. Wodejko-Kucharska, T. Saniewski, et al. Vitamin D level is associated with severity of coronary artery atherosclerosis and incidence of acute coronary syndromes in non-diabetic cardiac patients, Arch Med Sci, 15(2019):359-68.

17. N. Latic, R.G. Erben, Vitamin D and cardiovascular disease, with emphasis on hypertension, atherosclerosis, and heart failure, Int J Mol Sci, 21(2020):6483.

18. V.M.M. Giménez, L.G.A. Chuffa, V.A. Simão, R.J. Reiter, Protective actions of vitamin D, anandamide and melatonin during vascular inflammation: Epigenetic mechanisms involved, Life Sci, 288(2022):120191.

19. J. Monteiro, T. Souza, C. Curioni, C. Cople-Rodrigues, M. Citelli, Vitamin D and its effects on cell adhesion molecules: A systematic review, 2023.

20. A. Levin, G.L. Bakris, M. Molitch, M. Smulders, J. Tian, et al. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease, Kidney Int, 71(2007):31-8.

21. N. Chitalia, T. Ismail, L. Tooth, F. Boa, G. Hampson, et al. Impact of vitamin D supplementation on arterial vasomotion, stiffness and endothelial biomarkers in chronic kidney disease patients, PLoS One, 9(2014):e91363.