Ph.D. Dissertation of Sang-Ho Jo

Effects of Long-Term Vitamin C plus E Therapy on Vasospasm Improvement in Patients with Variant Angina

-ANOVA, a Prospective Randomized Clinical Trial-

이형 협심증 환자에서 항산화 비타민제제 (C+E)의 장기투여가 혈관경련 호전에 미치는 영향 연구: 아노바, 전향적 무작위 임상연구

February 2022

Graduate School of Medicine
Seoul National University
The interdisciplinary Program of Clinical Pharmacology

Sang-Ho Jo

Effects of Long-Term Vitamin C plus E Therapy on Vasospasm Improvement in Patients with Variant Angina

-ANOVA, a Prospective Randomized Clinical Trial-

Advisor: Hyo-Soo Kim

Submitting a Ph.D. Dissertation of
Medicine
October 2021

Graduate School of Medicine
Seoul National University
The interdisciplinary Program of Clinical Pharmacology
Sang-Ho Jo

Confirming the Ph.D. Dissertation written by
Sang-Ho Jo
January 2022

Chair	_____(Seal)
Vice Chair	_____(Seal)
Examiner	_____(Seal)
Examiner	_____(Seal)
Examiner	_____(Seal)

Abstract

Abstract

Background: Vitamin C and E as anti-oxidants have potential to improve vasospasm of coronary artery in patients with vasospastic angina (VA).

Methods: We enrolled prospectively VA patients with definite coronary vasospasm confirmed by coronary angiography (CAG) and ergonovine (EG) provocation test in single center. They were randomized to receive either vitamin C (ascorbic acid, 2g/day) plus E (tocopherol, 800 unit/day) (VITA) or control with underlying guideline-directed medical therapy for 2 years. Primary endpoint was change of minimal lumen diameter (MLD) at spasm site by EG provocation test at 2-year. MLD absolute value, percent diameter stenosis (DS), and DS change at 2-year were also compared in 2 groups. Composite clinical endpoint of death, acute myocardial infarction (AMI), revascularization and cardiac arrest was compared.

Results: Ninety six patients were diagnosed definite vasospasm defined as coronary narrowing ≥90% with concomitant chest pain or ECG change with EG provocation test. They were randomized to VITA (n=50) or control group (n=46). Baseline characteristics were similar in 2 groups. 85 and 86 spasm site (excluding left main coronary) of each group was analyzed. Provoked MLD change at 2 year and provoked absolute MLD at 2-year did not differ in VITA and non-VITA group, 0.14 ± 0.51 vs. 0.16 ± 0.34 mm respectively (P=0.777), and 0.33 ± 0.48 vs. 0.30 ± 0.32 mm respectively

(p=0.699). The DS change and DS at 2-year during EG provocation were also similar in both groups, -5.64 ± 17.20% vs. -5.58 ±15.08 respectively (p=0.984) and 86.9 ± 14.4% and 86.8 ± 13.6% in DS (p=0.970), respectively. Neither death nor AMI occurred. There was no difference in composite clinical endpoint, 4.3% in VITA and 6.0% in control group with majority of revascularization.

Conclusions: Vitamin C plus E did not influence on the spasm of coronary artery which is determined by provoked MLD and MLD change at 2year in patients with severe VA.

Key words: vasospastic angina, vitamin C, vitamin E, angiography, ergonovine provocation

Student Number: 2009-30595

Table of Contents

Chapter 1. Introduction

1.1. Study Background

Plausible mechanisms for coronary artery vasospasm in patient with vasospastic angina (VA) are hyperactive response of coronary vascular smooth muscle, vascular endothelial dysfunction, sympathetic overdrive and oxidative stress etc.[1-3] Although impairment of endothelium-medicated vasodilation which could be derived from common cardiovascular risk factors and atherosclerosis could in part associated with vasoconstriction at the site of predisposed segments, it is unlikely to be responsible for vasospasm by itself.[4] Rather, primary hyperreactivity of vascular smooth muscle cells has been suggested as playing a major role in VA development by consistent supporting evidences.[4,5] Among the triggers of vascular smooth muscle hyperreactivity, increased reactive oxygen species were suggested as in part being responsible.[6] Nonetheless for this, current mainstay of therapy for vasospasm is symptom management with calcium channel blockers and/or nitrate through coronary arterial smooth muscle relaxation causing vasodilaiton.[7-9] Considering the oxidative stress can be a trigger and is associated with vascular smooth muscle hyper-reactivity, targeting therapy on oxidative stress can be an alternative and potential fundamental treatment strategy other than conventional vasodilators.

Vitamin C and E are representative antioxidants and have myriad of data in experimental and clinical studies in various areas of cardiovascular

disease prevention, cancer prevention/treatment, pre-eclampsia prevention, fatty liver treatment, and sepsis.[10-17] The results were controversial and overall in failure including prevention cardiovascular events.

However there has been no randomized clinical trial to assess the efficacy of vitamin C and/or E in reducing vasospasm in patients with variant angina patients despite of its potential of endothelial function improvement of the vessel which are affected by oxidative stress.[18] If the study shows the positive results, antioxidant vitamin C and/or E could be another treatment modality for VA patients and can provide the insight of pathophysiology of vasospasm.

1.2. Purpose of Research

We performed prospective randomized open label clinical study-ANOVA (Effects of long term vitAmiN C plus E therapy on vasOspasm improvement and regression of atheroma in patients with VAriant angina) trial to evaluate the vitamin C plus E on the coronary spasm and natural course of VA patients with angiographic follow-up combined with EG provocation test.

Chapter 2. Body

2.1. Methods

Study design and population

ANOVA (effects of long term vitAmiN C plus E therapy on vasOspasm improvement and regression of atheroma in patients with VAriant angina) trial is an investigator-initiated, prospected, randomized, open-label, single-center trial performed at Seoul National University Hospital in South Korea. The study had two arms, (1) the normal control group and (2) the severe spasm group (Figure 1). The severe spasm group is randomized either into vitamin C+E treatment group (VITA) and control group. This study included only severe spasm group. After randomization, patients of vitamin group intake ascorbic acid 1g orally twice a day (2g/day) and tocopherol 400 IU orally twice a day (800 IU/day) for 2-years. Patients in control group do not intake study drugs. Patients in both groups received guideline directed medical therapy. Follow-up coronary angiography (CAG) and EG provocation test were performed 2 years after randomization.

Patients aged 19 years or older who received CAG and EG provocation test by physician's discretion with clinical suspicion of VA were eligible for this study. The exclusion criteria are as follows; (1) Patients with a significant fixed stenotic lesion in the main branch of the coronary artery after intracoronary nitroglycerin (NG) injection in CAG, (2) Patients who had continuously taken antioxidant vitamin complex within the past 3

months, (3) Female patients of childbearing age, (4) Patients whose survival is expected to be less than 1 year due to accompanying diseases, (5) Patients judged to be difficult to participate in this study due to other concomitant diseases.

The trial protocol was approved by the institutional review board at Seoul National University (IRB No. 1410-022-616). All patients provided written informed consent at the time of enrolment and randomization. This study was performed under the standards specified in the International Council for Harmonization Guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. This trial was registered at ClinicalTrials.gov, with ClinicalTrials.gov Identifier NCT03228238

Randomization

Immediately after the initial CAG and EG provocation test, eligible patients who showed severe coronary spasm were randomized 1:1 to either a vitamin complex intake group or a control group with open label. Eligible patients were randomized by block randomization method by an independent research nurse who was not involved in the trial.

Endpoints

The primary endpoint was minimal lumen diameter (MLD) change of provoked spam site at 2-year (provoked spasm site MLD at 2year- provoked spasm site MLD at 0year) between VITA and control groups. Absolute

MLD at 2-year, % diameter stenosis (DS) at 2 year and change of DS from index provocation test (provoked spasm site DS at 2year- provoked spasm site DS at 0year) were also compared in both groups. Change of coronary spasm provoked artery and coronary segment were also compared in 2 groups. Composite of clinical outcome of acute myocardial infarction, death, revascularization and cardia arrest was compared in VITA and control group at 2-year.

Coronary spasm provocation test

CAG was performed by routine manner and EG provocation test was performed following the Japanese Circulation Society guidelines.[19] By automated quantitative coronary angiography analysis (QCA), MLD and DS was measured after the EG provocation test as well as at pre-EG provocation (initial) and after nitroglycerin (NTG) bolus administration at index (0-year) and 2-year. A positive EG provocation test was defined as luminal narrowing ≥70% and an ischemic electrocardiogram (ECG) change and/or angina chest pain. Severe stenosis was defined as stenosis ≥ 90% by EG provocation test along with ischemic change of ECG and/or combined angina of the patient. If the patient's symptoms, ECG change, and hemodynamic profiles were tolerable, the route of administration and the maximum dose of EG were carried out following the same methods of the index test in follow-up EG provocation test.

Quantitative coronary angiography

QCA was performed at index test and at 2-year follow-up CAG at an independent core lab (Seoul National University Hospital Cardiovascular Clinical Research Center) by specialized technicians who were blinded to the allocation and purpose of the study. An offline computerized QCA system (CASS system; Pie Medical Instruments, Maastricht, the Netherlands) was used. Using the guiding catheter for magnification calibration, the minimal lumen diameters (MLD), percent diameter stenosis (DS), lesion length, and reference vessel diameters were measured from diastolic frames in a single, matched view showing the smallest MLD.

We focused on each spastic coronary artery segment. The coronary segments were coded according to the recommendations of the American Heart Association (AHA).[20] The most spastic segment was determined for each coronary artery during EG provocation. For more than 70% of spastic segments, QCA was performed at baseline, during EG provocation, and after intra coronary (IC) NTG administration. Matched spasm segment at index and at 2year provocation was compared for MLD and DS change calculation and if the spasm site moved or appeared or disappeared, the corresponding matched site was included in the analysis, for example, if proximal LAD segment spasm at index test moved to distal LAD segment, proximal segment at 2year was matched to original proximal LAD spasm and 2nd year distal LAD segment (moved at 2year) was matched to distal segment at 0 year.

Sample size estimation

The primary outcome is MLD change of spasm site at EG provocation test at 2-year as compared to that of index test. Because our study is concept proving trial, there was no previous study to refer for sample size estimation. Thus we adopt general concept of MLD change of ≥0.40mm as a reliable indicator of the presence or regression of coronary stenosis with QVA measurement.[21] Thus we assumed the VITA group will have improved MLD by 0.4mm and no change in that of non-VITA group, and standard deviation (SD) of VITA group as 0.55mm (arbitrarily estimated), so the expected effect size/SD (E/S) was calculated as 0.73 (0.40/0.55 mm). Thus 43 patients in each are needed to fulfill statistical power of 90% with type I error of 0.05 (2-sided). Assuming the lost to follow up rate as 15%, a total of 100 subjects (50 in each arm) was required.

Statistical analysis

Categorical data were compared with the chi-square or Fisher exact test as required in 2-groups. Continuous data are presented as mean ± SD and compared using the Student's t-test or the Mann–Whitney test. All analyses were performed using SPSS software version 25.0 (IBM Corporation, Armonk, NY) and R, version 3.5.2 (R foundation for Statistical Computing).

2.3. Results

Baseline characteristics and coronary arterial feature at 0- and 2-year

A total of 209 patients received coronary angiography (CAG) and EG provocation test. Among them, patients with severe vasospasm (n=96) were randomized to either vitamin C+E group (VITA, n=46) or control (non-VITA) group (n=50) (Figure 1). They all received follow-up CAG and EG provocation at 2-year. Clinical data was also collected. Baseline characteristic between VITA and non-VITA was similar (Table 1).

Spasm was provoked at 173 locations at first test. Spasm provoked site was majorly on right coronary artery (RCA) and left anterior descending artery (LAD), 42% (63/173) and 34.7% (60/173) followed by left circumflex artery (LCx), 22% (38/173). LM involvement was 1% (2/173). Proportion of LCx is higher in VITA group (Supplementary table 1).

MLD and DS at initial, EG provoked and after IC NTG in all spasm lesions (irrespective of coronary artery) were all comparable in VITA and non-VITA groups at 0- and 2-year. (Table 2)

Provoked spasm site absolute MLD and DS value at 2year and change during 2year by vitamin use or not

The MLD and DS after EG provocation were not different between VITA and non-VITA groups at 2 year in total coronary artery, 0.30 ± 0.32 mm vs. 0.33 ± 0.48 mm in MLD (p=0.699) and 86.9 ± 14.4% and 86.8 ± 13.6% in

DS (p=0.970), respectively (Table 3, Figure 2). In VITA group, the MLD was similar from mean 0.19 ± 0.21mm to 0.33 ± 0.45 mm (P=0.137) and DS significantly improved by EG provocation after 2 years as compared to that of baseline, from 92.42 ± 8.39 % to 86.78 ± 14.93 % (P=0.015) respectively.

In non-VITA group, the MLD and DS at EG provocation were also improved; MLD significantly increased from 0.17 ± 0.19 mm to 0.33 ± 0.33 mm (p=0.001) and DS significantly reduced from 91.56 ± 9.16% to 85.97 ± 14.09 % (p=0.003).

The magnitude of change of MLD and DS by EG provocation from baseline to 2 years were not different in VITA and non-VITA groups, 0.14 ± 0.51 vs. 0.16 ± 0.34 mm respectively (P=0.777) (table 3) in MLD and , -5.64 ± 17.20% vs. -5.58 ±15.08 respectively in DS (p=0.984) (table 3).

Change of spam severity for 2 years

Difference of provoked spasm site MLD and DS change as compared to those before EG provocation and post NG during 2 years was not different in 2 groups; [Change between initial and provoked MLD at 2year] minus [Change between initial and provoked at 0 year] for comparison between pre provocation and provocation; [difference between post NG and provoked MLD at 2year] minus [that between post NG and provoked at 0 year] for comparison between provocation and NTG administration. The ratio for those (2year data/0year data) was also similar (table 4).

Provoked spasm site MLD and DS at 2 year and their change as compared to those of 0year in individual coronary

In individual coronary artery, provoked MLD at LAD significantly less increased in VITA group as compared to non-VITA group, 0.02±0.26 vs. 0.21±0.35 mm (p=0.007) (table 5 [A]). The DS also decreased less in VITA group compared to control, -1.68±12.50 % and -8.76±13.19 % (p=0.021) respectively (table 5 [B]). The absolute value of provoked LAD MLD at 2-year was significantly lower in VITA group than in control, 0.17±0.29 vs. 0.39±0.29 (p=0.002). DS at 2-year was higher in VITA group than in control, 91.35±11.55 % vs. 83.88±12.48 %. (p=0.007) (table 5).

In RCA, provoked DS at 2-year was significantly lower in VITA group, 84.21±13.23% vs. 90.53±10.82 % (p=0.045) respectively (table 5 [B]). DS at provoked spasm site on RCA at 2year, the MLD change and DS change in RCA at 2-year from baseline were not different between the groups.

The MLD and DS at initial, during EG provocation test and after NG bolus administration were similar in both groups at 0-year and 2-year (Figure 3).

Spasm artery and segment change and development and disappearance of spasm during 2-years

During 2-year follow-up, spasm segment changed in 1/3 of spasm lesions. The segment change was more common in non-VITA group than in VITA group (Supplementary table 2, Supplementary table 3). More lesions of spasm disappeared in VITA group than in non-VITA but new spasm

developed with similar rate. (Supplementary table 2, Supplementary figure2).

Clinical outcomes

Total Death, acute myocardial infarction did not occur. One cardiac arrest occurred in control group and 4 revascularization (2 in VITA and 2 in control group) occurred. Composite clinical event of acute myocardial infarction, death, revascularization and cardiac arrest occurred similarly in both group, 4.3% (2/46) in VITA and 6.0% (3/50) in control group (P=1.000).

2.4. Discussions

We sought the effectiveness of long-term vitamin C plus E oral administration in the VA patients with repeated EG provocation test. The primary outcome of MLD change at 2-year confirmed by follow-up EG provocation test was similar in both groups. The DS change was also similar. The absolute value of MLD and DS at 2-year did not differ in both groups. In individual coronary analysis, in LAD, the MLD increment and DS decrement are more pronounced in control group, but, in RCA, DS were lower in vitamin users at 2-year. Overall MLD increased and DS decreased during 2-year irrespective of the vitamin use. Spasm segment changes commonly in 1/3 of all spasm lesions and more common in non-vitamin

users. Of note, disappearance of spasm was found in 1/5 lesions and more frequently found in VITA group. The clinical event occurs rarely and similar in both groups majorly driven by revascularization. Neither death nor MI was observed.

Our study results indicate that the treatment of vitamin C+E had no effect on coronary vasospasm by showing no difference in MLD/DS change and absolute value of them at 2year. Overall finding of increase of MLD and decrease of DS may affect the null effect of vitamin use. The relatively limited study population and dose not enough to full exertion of efficacy of the vitamin may be an explanation of negative results. However the dose used in this study (vitamin C of 2g/d, vitamin E of 800IU/d) could be appropriate as compared to previous dose of 0.5g-1.0mg of vitamin C and 400-800 IU of vitamin E oral formula for chronic use in large clinical trials.[11,14,22,23]

The change of spasm segment was observed in 50% (if newly appeared and disappeared site included) which is well coincide with previous reports of 46%.[24] Interestingly, the change was more common in non-VITA group and disappearance rate was more frequently found in VITA-group. This result might be caused by chance but the vitamin treatment could have a role.

Our study has value in its first randomization trial to test the efficacy of oral antioxidant vitamin C plus E in VA patients let alone to see the natural course of spastic coronary artery. Other study's strengths are (1) its large scale as compared to previous ones, (2) performing repeated CAG and EG

provocation test in all patients, (3) oral formula of vitamin C and E used, and (4) long-term oral intake of vitamins and long-term follow-up for 2-years.

Vitamin C and E have been tested with large scale clinical trials for various diseases including cardiovascular disease.[14] Despite of its theoretical benefit and evidences from observational studies with dietary form, prospective randomized trials with supplementation showed overall failure irrespective of its dose and target diseases.[11, 14, 23]

Among various target disease for vitamin treatment, there has been no clinical trials evaluating the efficacy of vitamins on patients with VA despite this drug has been suggested as having potential to improve the vascular endothelial function, ameliorate the activity of vascular smooth muscle cell tone and reducing the reactive oxygen species.[25-27] Hyper-reactivity of vascular smooth muscle cells has been suggested as main pathophysiologic mechanism for coronary vasospasm, but the cause of that feature was not fully elucidated.[4] Some triggers like inflammation, reactive oxygen free radicals and sympathetic over-drive were suggested.[4] The endothelial dysfunction was also regarded as one of the triggers of spasm, but not as major one causing vasospasm.[4] Some small studies demonstrated the reduced plasma level of vitamin E and/or C in those with vascular endothelial dysfunction including variant angina patients.[28, 29] Thus studies on the vasculature with vitamins were mainly focusing the endothelial dysfunction improvement assessed by the flow mediated vasodilation with

intravenous or arterial infusion of vitamins.[1, 18 26, 27] Other small sized clinical trial to see the spasm by intracoronary administration of vitamin C showed the potential of that drug. However this study employed the methods of intracoronary bolus administration one time and the purpose was not to see chronic change of spasm by repeated EG provocation with long term intake of vitamin albeit of its difficulty in applying to clinical practice.[27]

Our study objective is to seek the fundamental treatment modality of VA patients by changing the spastic nature of coronary vasculature by chronic supply of antioxidant. Although, we failed in proving the improvement of spasm with long term oral intake of vitamin C and E, our study has meaning in its proof of concept nature and provide the evidence of futility of these drug in vasospasm in VA patients. Also this study provided the failure of changing the innate nature vasculature of patients with VA.

Some positive signals for future investigation was detected; more disappearance of spasm and no change of spasm segment in vitamin users were found. This finding and the meaning ought to be investigated further.

2.5. Limitations

Firstly, the dose of vitamin could be not enough to show the efficacy of antioxidant. The serum level on that could help estimate the adequacy of the dose. Secondly the sample size might be small to see the difference. Thirdly,

compliance data on the study drug did not presented.

Chapter 3. Conlusions

Vitamin C plus E treatment did not reduce coronary spasm assessed by MLD and DS change in patients with severe VA in 2-year follow-up EG provocation test.

Bibliography

1. Hamabe A, Takase B, Uehata A, Kurita A, Ohsuzu F, Tamai S. Impaired endothelium-dependent vasodilation in the brachial artery in variant angina pectoris and the effect of intravenous administration of vitamin C. Am J Cardiol 2001;**87**(10):1154-9.

2. Motoyama T, Kawano H, Kugiyama K, Okumura K, Ohgushi M, Yoshimura M, Hirashima O, Yasue H. Flow-mediated, endothelium-dependent dilatation of the brachial arteries is impaired in patients with coronary spastic angina. Am Heart J 1997;**133**(3):263-7.

3. Ong P, Athanasiadis A, Borgulya G, Mahrholdt H, Kaski JC, Sechtem U. High prevalence of a pathological response to acetylcholine testing in patients with stable angina pectoris and unobstructed coronary arteries. The ACOVA Study (Abnormal COronary VAsomotion in patients with stable angina and unobstructed coronary arteries). J Am Coll Cardiol 2012;**59**(7):655-62.

4. Lanza GA, Careri G, Crea F. Mechanisms of coronary artery spasm. Circulation 2011;**124**(16):1774-82.

5. Kaski JC, Tousoulis D, Gavrielides S, McFadden E, Galassi AR, Crea F, Maseri A. Comparison of epicardial coronary artery tone and reactivity in Prinzmetal's variant angina and chronic stable angina pectoris. Journal of the American College of Cardiology 1991;**17**(5):1058-1062.

6. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. Hypertens Res 2011;**34**(4):431-40.

7. Kim SE, Jo SH, Han SH, Lee KY, Her SH, Lee MH, Seo WW, Cho SS, Baek SH. Comparison of calcium-channel blockers for long-term clinical outcomes in patients with vasospastic angina. Korean J Intern Med 2021;**36**(1):124-134.

8. Kook H, Hong SJ, Yang KS, Lee S, Kim JS, Park CG. Comparison of nebivolol versus diltiazem in improving coronary artery spasm and quality of life in patients with hypertension and vasospastic angina: A prospective, randomized, double-blind pilot study. PLoS One 2020;**15**(9):e0239039.

9. Parker JD, Parker JO. Nitrate therapy for stable angina pectoris. N Engl J Med 1998;**338**(8):520-31.

10. Sevransky JE, Rothman RE, Hager DN, Bernard GR, Brown SM, Buchman TG, Busse LW, Coopersmith CM, DeWilde C, Ely EW, Eyzaguirre LM, Fowler AA, Gaieski DF, Gong MN, Hall A, Hinson JS, Hooper MH, Kelen GD, Khan A, Levine MA, Lewis RJ, Lindsell CJ, Marlin JS, McGlothlin A, Moore BL, Nugent KL, Nwosu S, Polito CC, Rice TW, Ricketts EP, Rudolph CC, Sanfilippo F, Viele K, Martin GS, Wright DW. Effect of Vitamin C, Thiamine, and Hydrocortisone on Ventilator- and Vasopressor-Free Days in Patients With Sepsis: The VICTAS Randomized Clinical Trial. Jama 2021;**325**(8):742-750.

11. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;**362**(18):1675-85.

12. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet 2003;**361**(9374):2017-23.

13. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. Jama 2005;**294**(1):56-65.

14. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, Bubes V, Manson JE, Glynn RJ, Gaziano JM. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. Jama 2008;**300**(18):2123-33.

15. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. N Engl J Med 1985;**312**(3):137-41.

16. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS.

Vitamins C and E and the risks of preeclampsia and perinatal complications. N Engl J Med 2006;**354**(17):1796-806.

17. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med 2001;**345**(22):1583-92.

18. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K. Endothelial function and oxidative stress in renovascular hypertension. N Engl J Med 2002;**346**(25):1954-62.

19. Guidelines for diagnosis and treatment of patients with vasospastic angina (Coronary Spastic Angina) (JCS 2013). Circ J 2014;**78**(11):2779-801.

20. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, McGoon DC, Murphy ML, Roe BB. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. Circulation 1975;**51**(4 Suppl):5-40.

21. Waters D, Lespérance J, Craven TE, Hudon G, Gillam LD. Advantages and limitations of serial coronary arteriography for the assessment of progression and regression of coronary atherosclerosis. Implications for clinical trials. Circulation 1993;**87**(3 Suppl):Ii38-47.

22. McCance DR, Holmes VA, Maresh MJ, Patterson CC, Walker JD, Pearson DW, Young IS. Vitamins C and E for prevention of pre-eclampsia in women with type 1 diabetes (DAPIT): a randomised placebo-controlled trial. Lancet 2010;**376**(9737):259-66.

23. Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, Pearson GD, Wapner RJ, Varner MW, Thorp JM, Jr., Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Harper M, Smith WJ, Saade G, Sorokin Y, Anderson GB. Vitamins C and E to prevent complications of pregnancy-associated hypertension. N Engl J Med 2010;**362**(14):1282-91.

24. Ozaki Y, Keane D, Serruys PW. Fluctuation of spastic location in patients with vasospastic angina: a quantitative angiographic study. J Am Coll Cardiol 1995;**26**(7):1606-14.

25. Siow RC, Richards JP, Pedley KC, Leake DS, Mann GE. 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 1999;**19**(10):2387-94.

26. Motoyama T, Kawano H, Kugiyama K, Hirashima O, Ohgushi M, Tsunoda R, Moriyama Y, Miyao Y, Yoshimura M, Ogawa H, Yasue H. Vitamin E administration improves impairment of endothelium-dependent vasodilation in patients with coronary spastic angina. J Am Coll Cardiol 1998;**32**(6):1672-9.

27. Kugiyama K, Motoyama T, Hirashima O, Ohgushi M, Soejima H, Misumi K, Kawano H, Miyao Y, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Yasue H. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1998;**32**(1):103-9.

28. Miwa K, Miyagi Y, Igawa A, Nakagawa K, Inoue H. Vitamin E deficiency in variant angina. Circulation 1996;**94**(1):14-8.

29. Miwa K, Igawa A, Nakagawa K, Hirai T, Inoue H. Consumption of vitamin E in coronary circulation in patients with variant angina. Cardiovasc Res 1999;**41**(1):291-8.

Figure Legends

Figure 1. Study flow

Figure 2. Change of provoked site MLD during 2 years in VITA and non-VITA groups.

Figure 3. (A) MLD and (B) DS in initial, EG provocation and after NTG bolus administration in VITA vs. non-VITA groups at 2year
*MLD, minimal lumen diameter; DS, diameter stenosis; Prov, EG-provocation; NTG, nitroglycerin

Abstract

배경: 비타민 C와 E는 항산화제로서, 이형협심증 환자의 관상동맥 연축을 개선시킬 가능성이 있다.

방법: 관상동맥 조영술과 에르고노빈 연축 검사를 통하여 진단된 확실한 이형협심증환자를 단일 기관에서 모집하였다. 환자들은 비타민 C (아스코빅산 2g/d)와 비타민 E(tocopherol 800unit/d) 의 복합제 복용군 (VITA) 혹은 대조군 (non-VITA)으로 무작위 배정되었다. 양군은 모두 표준 치료를 받았다. 일차 목표 변수는 2년후 관상동맥 조영술과 에르고노빈 연축 검사를 통한 관상동맥 최소내강 직경 (minimal lumen diameter [MLD])으로 하였다. 2년 추적 연축 검사시의 MLD 수치와 percent diameter stenosis (DS), DS 변화도 관찰하고 양군에서 비교하였다. 사망, 급성심근경색증, 재관류화, 심정지의 복합 임상 사건도 양군에서 비교하였다.
결과: 혈관 연축 검사에서 내경이 90%이상 협착이 오고 동시에 흉통이나 심전도의 변화가 있는 96명의 확실한 이형협심증 환자를 모집하였다. 그들은 VITA 군(50명) 혹은 대조군 (46명)으로 무작위 배정되었다. 기저 특징은 양군에 유사하였다. 양군에서 각각 85개, 86개의 병변이 비교되었다 (좌주간지제외). 2년후 연축 부위의, 2년간의 MLD 변화와 2년 MLD자체는, 각각 0.14 ± 0.51 vs. 0.16 ± 0.34 mm (p=0.777) 와 0.33 ± 0.48 vs. 0.30 ± 0.32 mm (p=0.699) 로서 양군에서 차이가 없었다. DS 의 변화와 DS의 절대 수치도, -5.64±17.20 vs. -5.58±15.08% (p=0.984) 와 86.9 ± 14.4% vs. 86.8 ± 13.6%로 양군에서 차이가 없었다. 임상 사건에서도 각각 4.3% (2/46) (VITA) 와 6.0% (3/50) (대조군)로 차이가 없었다. 사망과 심근경색증은 발생하지 않았고 주로 재관류화가 주된 사건이었다.
결론: 비타민 C+E의 장기간 복합 요법은, 연축 부위 MLD와 MLD 변화로 본 이형협심증환자의 관상동맥 연축 정도에 영향을 끼치지 못하였다.

중심단어: 혈관 연축성 협심증, 비타민 C, 비타민 E, 관상동맥 조영술, 에르고노빈 유발 검사

Tables 1. Baseline characteristics

Variables	**Total (n=96)**	**Vitamin C+E (n=46)**	**NO-Vitamin (n=50)**	**P-value**
Sex, n (%)				
Male	80(83.0)	38(82.6)	42(84.0)	0.855
Female	16(16.7)	8(17.4)	8(16.0)	
Age (Mean, SD), n (%)				
≥ 65 years old, n (%)	43(44.8)	22(47.8)	21(42.0)	0.566
< 65 years old, n (%)	53(55.2)	24(52.2)	29(58.0)	
BMI (Mean, SD)	25.16±2.87	24.82±3.01	25.47±2.73	0.274
Disease history, n (%)				
HTN	53(55.2)	23(50.0)	30(60.0)	0.325
DM	15(15.6)	6(13.0)	9(18.0)	0.504
Dyslipidemia	79(82.3)	38(82.6)	41(82.0)	0.938
Stroke	6(6.3)	1(2.2)	5(10.0)	0.206
CKD	1(1.0)	0(0.0)	1(2.0)	1.0
LVEF	59.68±5.95	58.23±6.97	60.62±5.08	0.118
PCI history, n	13(13.5)	7(15.2)	6(12.0)	0.645
CABG history, n	0(0.0)	0(0.0)	0(0.0)	-
Myocardial infarction history	0(0.0)	0(0.0)	0(0.0)	-
Heart failure history	0(0.0)	0(0.0)	0(0.0)	-

Hb	14.12±1.45	14.10±1.36	14.13±1.55	0.937
WBC	6.79±1.91	6.49±1.30	7.06±2.32	0.141
Platelet	224.62±57.05	215.69±60.85	232.84±52.60	0.142
Fibrinogen	309.71±82.70	311.44±71.77	308.14±92.14	0.847
Serum creatinine, mg/dL	0.97±0.22	0.98±0.20	0.97±0.25	0.878
Potassium,	4.10±0.36	4.13±0.40	4.07±0.31	0.442
Total cholesterol, (Mean, SD)	154.85±32.29	154.30±33.83	155.37±31.12	0.874
TG (Mean, SD)	145.73±106.96	128.07±61.98	164.21±137.80	0.114
HDL-C(Mean, SD)	44.98±10.62	43.78±9.71	46.13±11.41	0.287
LDL-C (Mean, SD)	88.03±30.38	90.11±31.68	86.04±29.29	0.524
Fasting glucose (Mean, SD)	120.03±34.68	121.00±33.40	119.02±36.34	0.791
HbA1C	6.13±1.40	6.28±1.88	5.99±0.68	0.441
CRP	1.64±10.71	0.62±1.54	2.52±14.58	0.413
Medications, n (%)				
aspirin	56(58.3)	25(54.3)	31(62.0)	0.447
clopidogrel	19(19.8)	9(19.6)	10(20.0)	0.957
ARBs	13(13.5)	3(6.5)	10(20.0)	0.054
ACEIs	2(2.1)	1(2.2)	1(2.0)	1.0
Beta-blockers	2(2.1)	2(4.3)	0(0.0)	0.227
CCBs	76(79.2)	33(71.7)	43(86.0)	0.086
molsidormin	33(34.4)	17(37.0)	16(32.0)	0.609

nicorandil		52(54.2)	31(67.4)	21(42.0)	**0.013**
nitrate		73(76.0)	34(73.9)	39(78.0)	0.639
trimetazidine		16(16.7)	10(21.7)	6(12.0)	0.201
EG dose at 0yr		IV, mcg	569.57 ± 119.01 (n=46)	570.00±117.43 (n=20)	569.23±122.54 (n=26)
EG dose at 0yr	IC, mcg	85.00 ± 22.58 (n=6)	90.0 (n=2)	82.50 ± 28.72 (n=4)	0.983
EG dose at 2yr	IV, mcg	544.19 ±109.77 (n=43)	522.22 ±100.33 (n=18)	560.00 ± 115.47 (n=25)	0.800
EG dose at 2yr	IC, mcg	84.00 ± 25.10 (n=5)	90.00 ± 30.00 (n=3)	75.00 ± 21.21 (n=2)	0.271

*BMI, body mass-index; HTN, hypertension; DM, diabetes mellitus; CKD, chronic kidney disease; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; CABG, coronary arterial bypass graft; Hb, hemoglobin; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; ARBs, angiotensin receptor blockers; ACEIs, angiotensin-converting enzyme inhibitors; CCBs, calcium channel blockers; EG, ergonovine

Table 2. All spasm lesion comparison between VITA and non-VITA groups

All lesion	VITA (n = 54)	Non-VITA (n =47)	p-value
At initial			
initial RD, mm	2.41 ± 0.60	2.45 ± 0.43	0.751
initial MLD, mm	1.40 ± 0.71	1.52 ± 0.44	0.412
initial DS,%	43.46 ± 21.21	39.49 ± 19.67	0.402
Prov-RD, mm	2.21 ± 0.49	2.07 ± 0.64	0.291
Provo-MLD, mm	0.18 ± 0.19	0.15 ± 0.18	0.507
Provo-DS, %	92.83 ± 7.76	91.66 ± 9.18	0.488
NG-RD, mm	2.21 ± 0.049	2.07 ± 0.64	0.291
NG-MLD, mm	2.59 ± 0.47	2.67 ± 0.47	0.445
NG-DS, %	16.25 ± 13.47	13.07 ± 8.53	0.189
At 2-Year Follow-up			
initial RD, mm	2.26 ± 0.54	2.34 ± 0.42	0.496
initial MLD, mm	1.29 ± 0.51	1.39 ± 0.46	0.374
initial DS, %	42.62 ± 18.21	40.39 ± 16.65	0.583
Provo-RD, mm	2.10 ± 0.49	2.09 ± 0.47	0.942
Provo-MLD, mm	0.29 ± 0.41	0.36 ± 0.34	0.426
Provo-DS, %	86.85 ± 14.42	84.17 ± 15.45	0.372
NTG-RD, mm	2.60 ± 0.54	2.71± 0.56	0.398
NTG-MLD, mm	2.09 ± 0.54	2.22 ± 0.52	0.283
NTG-DS, %	19..54 ±11.85	18.03 ± 9.64	0.545

**RD, reference vessel diameter; MLD, minimal lumen diameter; DS, diameter stenosis; Prov, EG-provocation; NTG, nitroglycerin

Table 3. Provoked spasm site MLD/DS change (provoked MLD/DS at 2year - provoked MLD/DS at 0year) and absolute MLD/DS value at 2year between VITA and non-VITA in all matched segment of coronary arteries (spasm newly appeared and disappeared included)

	VITA (n = 96)	Non-VITA (n = 98)	P value
Provoked-MLD change (provoked MLD at 2yr- provoked MLD at 0yr)	0.14 ± 0.51	0.16 ± 0.34	0.777
Provoked-MLD at 2yr	0.30 ± 0.32	0.33 ± 0.48	0.699
Provoked-DS change (%) (provoked DS at 2yr- provoked DS at 0yr)	-5.64 ± 17.20	-5.58 ± 15.08	0.984
Provoked-DS at 2 year (%)	86.89 ± 14.38	86.81 ± 13.61	0.970

* Mann-whitney U-test

MLD, minimal lumen diameter; DS, diameter stenosis

Table 4. Provoked spasm site MLD/DS at 2year and provoked MLD change (provoked MLD/DS at 2year – provoked MLD/DS at 0year) between VITA and non-VITA groups at matched segment of individual coronary arteries (spasm newly appeared and disappeared included)

(A) MLD

	VITA	Non-VITA	P -value*
Prov. LAD MLD at 2yr	0.17 ± 0.29 (n= 19)	0.39 ± 0.29 (n =24)	0.002
LAD MLD change	0.02 ± 0.26 (n=18)	0.21 ± 0.35 (n = 19)	0.007
Prov. LCX MLD at 2yr	0.38 ± 0.62 (n= 14)	0.32 ± 0.42 (n =12)	0.781
LCx MLD change	0.35 ± 0.77 (n=10)	0.22 ± 0.49 (n =10)	0.853
Prov. RCA MLD at 2yr	0.41 ± 0.51 (n=25)	0.22 ± 0.28 (n = 31)	0.118
RCA MLD change	0.14 ± 0.50 (n=20)	0.10 ± 0.27 (n=25)	0.766

* Mann-whitney U-test

(B) DS

	VITA (+)	Non-VITA	P -value*
Prov. LAD DS at 2yr	91.35 ± 11.55 (n =26)	83.88 ± 12.48 (n=32)	0.007
Prov. LAD DS change	-1.68 ± 12.50 (n=22)	-8.76 ± 13.19 (n =25)	0.021
Prov. LCX DS at 2yr	85.00 ± 18.16(n=20)	83.43 ± 20.10 (n=14)	0.986
LCx DS change at 2 yr	-10.76 ± 21.91(n=20)	-8.83 ± 26.33 (n=14)	0.556
Prov. RCA DS at 2yr	84.21 ± 13.23 (n =29)	90.53 ± 10.82 (n = 38)	0.045
RCA DS change	-5.64 ± 16.94 (n= 25)	-2.2 ± 10.24 (n = 35)	0.279

* Mann-whitney U-test

LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; MLD, minimal lumen diameter; DS, diameter stenosis; Prov, EG-provocation; NTG, nitroglycerin

Table 5. Spasm site MLD/DS difference (as compared to that of initial and post NTG) change and MLD difference ratio from 0year to 2year between VITA and non-VITA in (spasm newly appeared and disappeared included)

	VITA (n = 96)	Non-VITA (n = 98)	p value
Difference			
2yr MLD delta (initial-prov)-0yr MLD delta (initial-prov)	-2.26 ± 1.08	-2.51 ± 0.77	0.185
2yr MLD delta (NTG-prov)-0yr MLD delta(NTG-prov)	-0.30 ± 0.76	-0.34 ±0.54	0.461
2y DS (prov-initial) delta – base DS (prov-initial)delta	-4.48 ± 24.86	-8.76 ± 25.58	0.575
2y DS (prov-NTG) delta – base DS (prov-NTG)delta	-6.49 ± 22.48	-11.28 ± 18.26	0.096
Ratio			
2yr MLD delta (initial-prov) /0yr MLD delta (initial-prov)	0.97 ± 0.73	0.95 ± 0.96	0.793
2yr MLD delta (NTG-prov)/ 0yr MLD delta(NTG-prov)	0.92 ± 0.37	0.85 ± 0.25	0.305

[§]Mann-whitney U-test; MLD, minimal lumen diameter; NTG, nitroglycerin, prov, provocation

Figure 1.

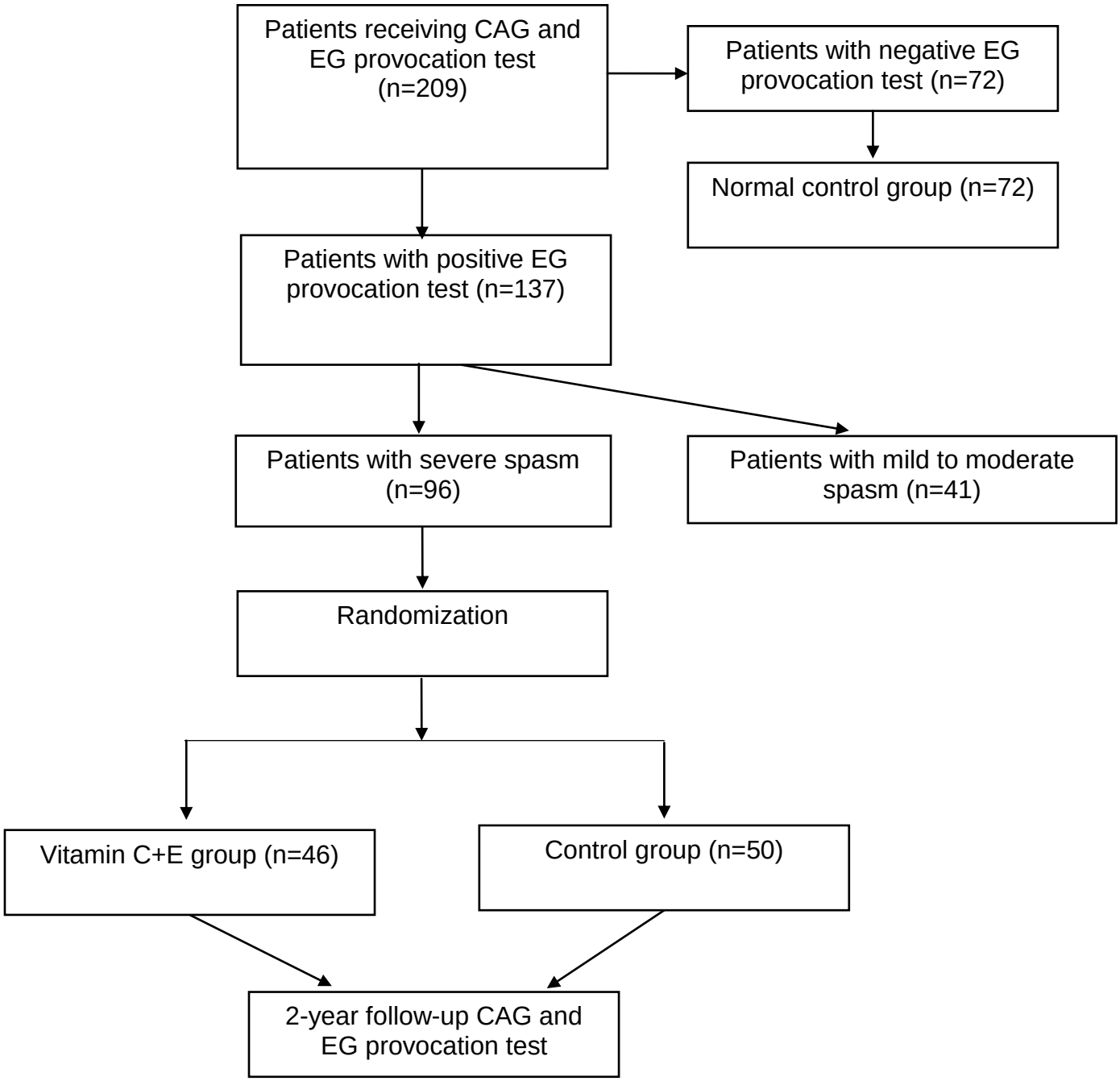

Patients receiving CAG and EG provocation test (n=209)
Patients with negative EG provocation test (n=72)
Normal control group (n=72)
Patients with positive EG provocation test (n=137)
Patients with severe spasm (n=96)
Patients with mild to moderate spasm (n=41)
Randomization
Vitamin C+E group (n=46)
Control group (n=50)
2-year follow-up CAG and EG provocation test

Figure 2.

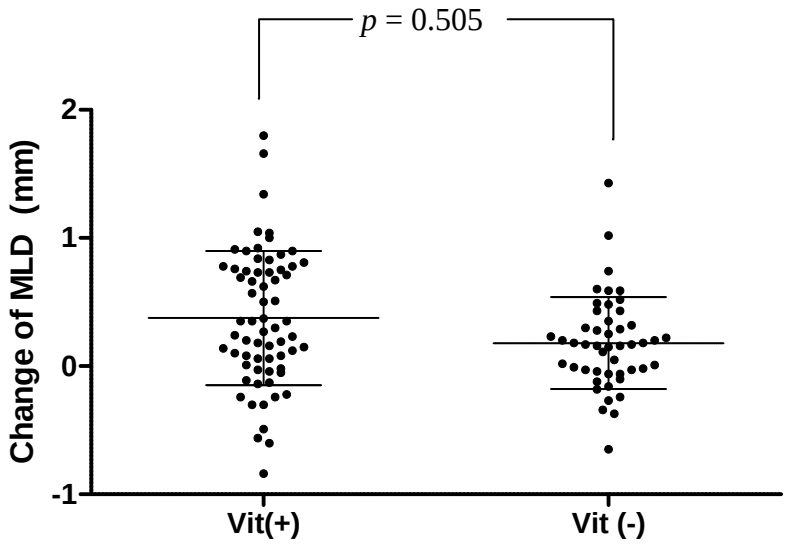
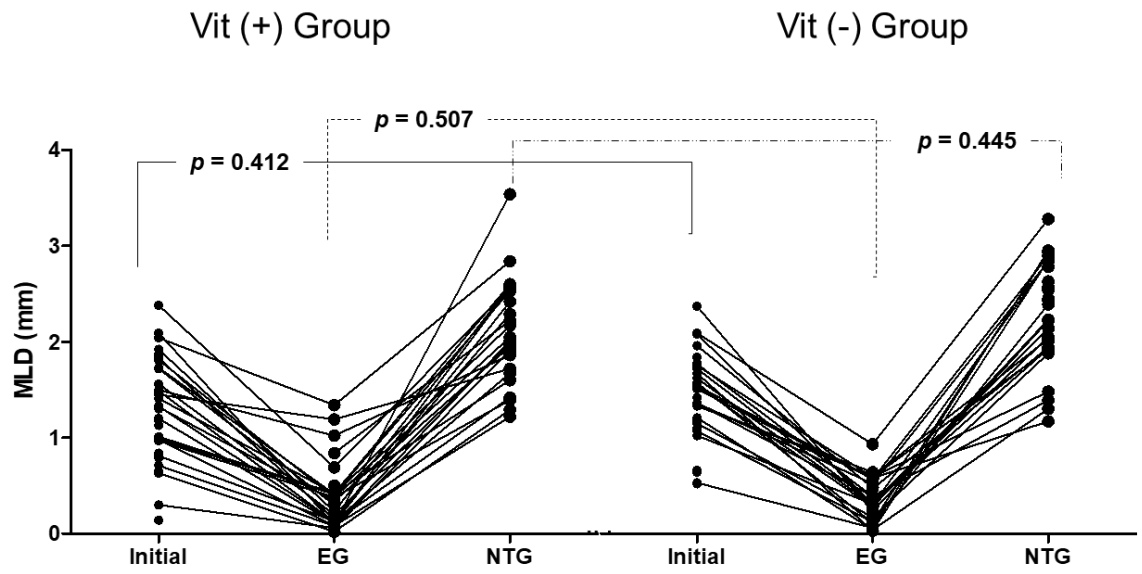
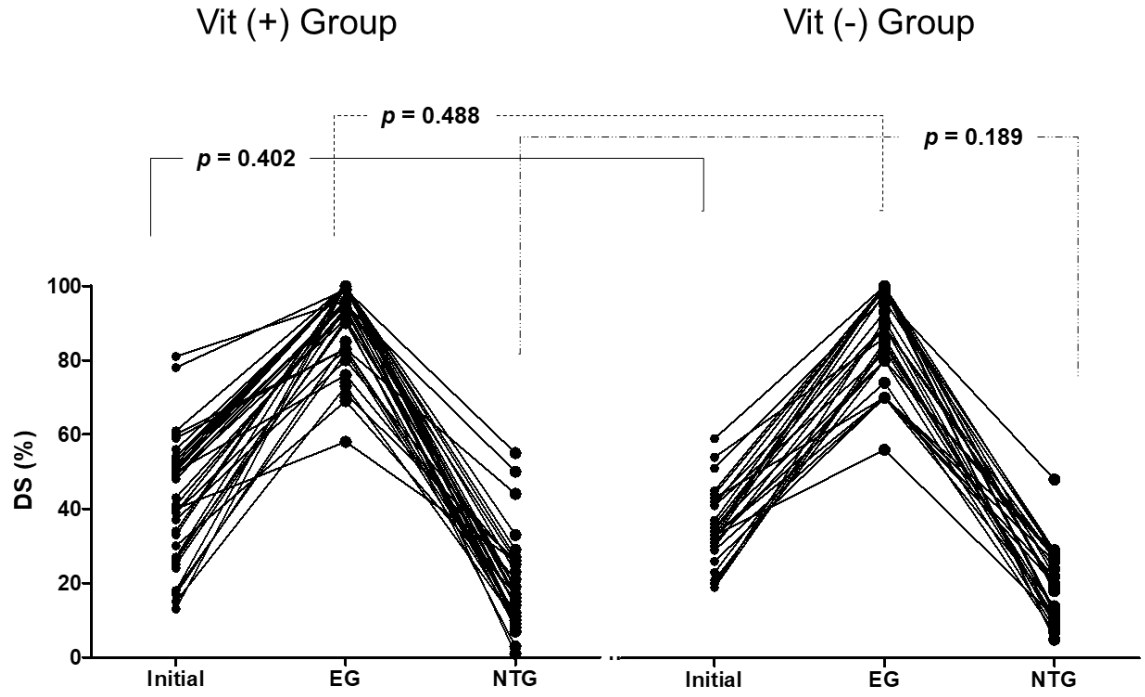


Figure 3.

(A)


Vit (+) Group
Vit (-) Group
p = 0.507
p = 0.412
p = 0.445
MLD (mm)
0
1
2
3
4
Initial
EG
NTG
Initial
EG
NTG

(B)


Vit (+) Group
Vit (-) Group
p = 0.488
p = 0.402
p = 0.189
DS (%)
0
20
40
60
80
100
Initial
EG
NTG
Initial
EG
NTG

Supplementary materials

Supplementary Table 1.

(A) Distribution of spasm lesion (no.) at 0 year

	VITA (+)	Non-VITA (-)	P -value
spasm at LAD, lesion no. (%)	28 (60.9)	32 (64.0)	0.752
spasm at LCx, lesion no. (%)	23 (50.0)	15 (30.0)	0.045
spasm at RCA, lesion no. (%)	33 (71.7)	39 (78.0)	0.502
spasm at LMCA, lesion no. (%)	0 (0.0)	2 (4.0)	0.496
Total no. %	84, 100%	88, 100%	

(B) Distribution of spasm lesion at 2 year (n)

	VITA (+)	Non-VITA	P -value
spasm at LAD, lesion no, (%)	27(58.7)	32(64.0)	0.594
spasm at LCx, lesion no, (%)	19(41.3)	14(28.0)	0.170
spasm at RCA, lesion no, (%)	29(63.0)	38(76.0)	0.167
spasm at LMCA, lesion no, (%)	0 (0.0)	1 (2.0)	1.000
Total no., %	75, 100%	85, 100%	

Supplementary Table 2. Spasm segment change according to vitamin use or not

(A) Excluding spasm appearance or disappearance

0yr ➔ 2yr	All (n = 137)	VITA (n=65)	Non-VITA(n=72)	p-value
Spasm segment site same	101 (73.7)	54 (83.1)	47 (65.3)	0.018
Spasm segment site change	36 (26.3)	11 (16.9)	25 (34.7)	-

(B) Including spasm appearance or disappearance

0yr ➔ 2yr	All (n= 194)	VITA(n= 96)	Non-VITA (n=98)	p-value
Spasm segment same	101 (52.1)	54 (56.3)	47 (48.0)	0.072
Spasm segment changed	36 (18.6)	11 (11.5)	25 (25.5)	-
Spasm site disappear	34 (17.5)	20 (20.8)	14 (14.3)	-
Newly detected spasm site	23 (11.9)	11 (11.5)	12 (12.2)	

Supplementary Table 3. Spasm segment status during 2 years in individual coronary artery

(A) LAD (Not changed 36, changed 11, disappeared 13, appeared 11)

Spasm segment unchanged	n
pLAD → pLAD	11
mLAD → mLAD	20
dLAD → dLAD	2
Diagonal → Diagonal	3
Spasm segment changed	
pLAD → mLAD	5
mLAD → pLAD	2
mLAD →Diagonal	2
Diagonal → pLAD	1
Septal → mLAD	1

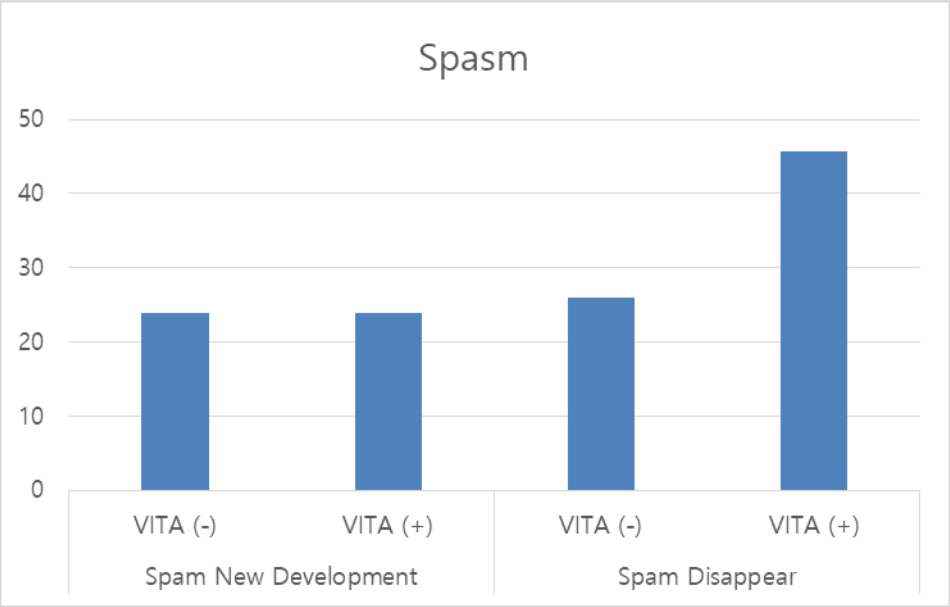
(B) LCx (Not changed 24, changed 5, disappeared 10, appeared 5)

Spasm segment unchanged	n
pLCx → pLCx	10
dLCx → dLCx	10
OM → OM	4
Spasm segment changed	
pLCx → OM	1
dLCx → OM	3
OM → dLCx	1

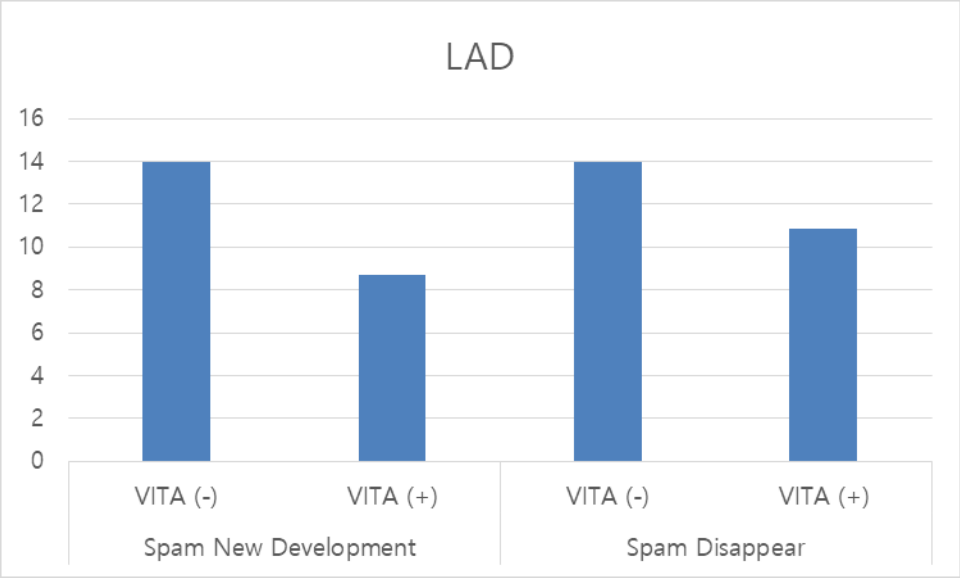
(C) RCA (Not changed 41, changed 20, disappeared 11, appeared 7)

Spasm segment unchanged	n
pRCA → pRCA	8
mRCA → mRCA	20
dRCA → dRCA	8
PL → PL	3
PDA → PDA	2
pRCA → mRCA	5
pRCA → dRCA	1
pRCA → PL	1
mRCA → pRCA	4
mRCA → dRCA	2
dRCA →pRCA	1
dRCA → mRCA	3
PL → dRCA	1
PL → PDA	1
PDA → mRCA	1

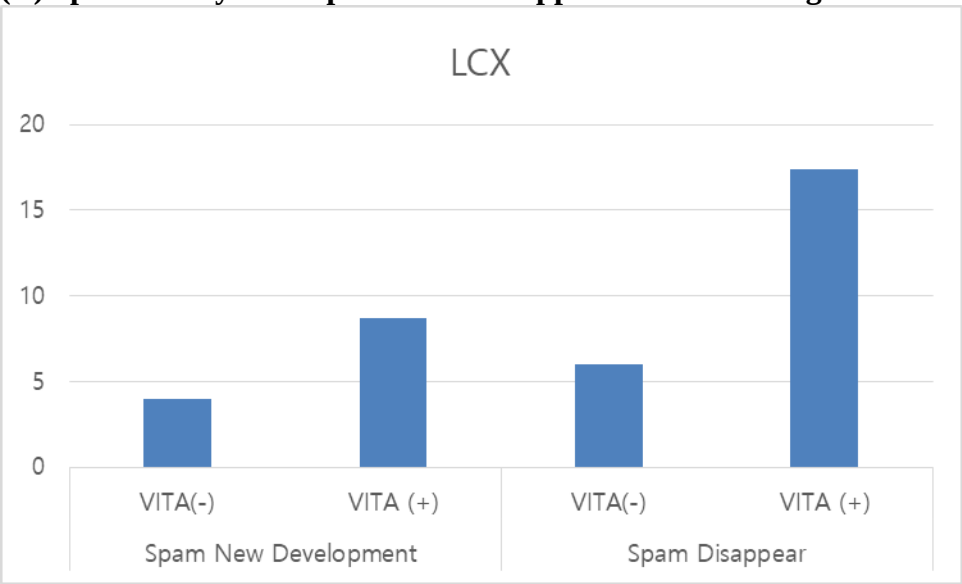
Supplementary figure 1 (A) Spasm newly development and disappearance according to vitamin use, overall



(B) Spasm newly development and disappearance according to vitamin use in LAD



(C) Spasm newly development and disappearance according to vitamin use in LCx



(D) Spasm newly development and disappearance according to vitamin use in RCA