Vitamin E and antioxidant activity; its role in slow coronary flow

VEYSEL KENAN CELIK, İMGE EZGI EKEN, GÜRSEL YILDIZ, MEHMET BIRHAN YILMAZ, AHMET GURLEK, HÜSEYIN AYDIN

Abstract

Aim: Oxidative stress, which is widely recognised as an important feature of many diseases, can be defined as an increased formation of reactive oxygen species or decreased antioxidant defense. In this study we measured plasma vitamin E levels and total antioxidant activity (AOA) in patients with slow coronary flow (SCF).

Methods: The plasma vitamin E levels and AOA were measured in 40 patients with angiographically diagnosed SCF. Forty subjects with normal coronary flow (NCF) served as the control group. SCF and NCF were analysed, and blood samples were taken for plasma vitamin E levels and AOA. Plasma vitamin E levels and AOA in patients with SCF were evaluated and compared to those of patients with NCF.

Results: There was no significant difference between the two groups in terms of plasma AOA, lipid profile and C-reactive protein (CRP) levels but there was a significant difference in vitamin E levels between the two groups (p = 0.001).

Conclusion: Vitamin E levels were found to be lowered in patients with SCF compared to the NCF group. The association between smoking and vitamin E levels is worth further investigating in larger samples.

Keywords: vitamin E, antioxidant activity, slow coronary flow

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The ability of antioxidant defense to scavenge reactive oxygen species (ROS) is important to protect tissues from oxidative damage. Cells and biological fluids have an array of protective antioxidant mechanisms, enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic, referred to as chain-breaking antioxidants (tocopherols, ubiquinol, carotenoids and flavonoids as lipid phase, and ascorbate, urate, glutathione and other thiols as aqueous phase), both for preventing the production of free radicals and for repairing oxidative damage."""" A free radical contains an unpaired electron in an atomic orbital. In this state, no molecular species is stable for long. A free radical will attract other molecules and either give or receive an electron to make itself thermodynamically stable. The most important free radicals in many disease states are oxygen derivatives such as hydrogen peroxide, superoxide and particularly, hydroxyl radical, which is the most harmful for tissues. Transition metals contain one or more unpaired electrons and are therefore also radicals when in the elemental state. However, their key property from the point of view of free radical biology is their variable valency, which allows them to undergo reactions involving the transfer of a single electron. The most important transition metals in human disease are iron and copper. These elements play a key role in the production of hydroxyl radicals \textit{in vivo}.

Hydrogen peroxide and superoxide can be detoxified enzymatically in the mammalian system by catalase and superoxide dismutase, respectively. However there is no enzymatic system that converts or detoxifies hydroxyl radicals. A hydroxyl radical can be detoxified by non-enzymatic systems. One of these is the tocopherols (\textalpha{}, \textbeta{}, \textgamma{} and \textdelta{}), which have a chromanol ring and a phenyl tail, and differ in the number and position of the methyl groups on the ring. The most important lipid-phase antioxidant is probably vitamin E."

The coronary slow-flow phenomenon was first described in 1972 by Tambe et al. \textsuperscript{14} The phenomenon is an angiographic finding characterised by delayed distal vessel opacification in the absence of significant epicardial coronary disease. However, since that time, only a limited number of studies have focused on the aetiology of this unique angiographic phenomenon.

Histopathological studies have revealed the loss of luminal diameter, and capillary and endothelial damage in these patients.\textsuperscript{15} Although the pathophysiological mechanisms of slow coronary flow phenomenon remain uncertain, there are several hypotheses that have been suggested, including endothelial activation and inflammation.\textsuperscript{11} However, the phenomenon is not well studied and deserves further investigation.

In the present study, we investigated plasma vitamin E levels and antioxidant activity in patients with slow coronary flow (SCF) and compared them with those with normal coronary flow (NCF).}

Methods

Forty consecutive patients with angiographically diagnosed SCF in all three epicardial coronary arteries, and 40 subjects with normal coronary flow as a control group were enrolled in our study after obtaining informed consent. All patients underwent selective coronary angiography via the Judkins technique.
Coronary flow of both groups was documented by the thrombolysis in myocardial infarction (TIMI) frame count (TFC). The TIMI frame count method is a simple, reproducible and quantitative index of coronary flow.\textsuperscript{14} To obtain corrected TFC for the left anterior descending (LAD) coronary artery, the TIMI frame count was divided by 1.7. The mean TFC for both groups was calculated by adding the TFC for the LAD, left circumflex artery (LCx) and right coronary artery (RCA), and then dividing the obtained value by three.

The TFC in the LAD and the LCx were assessed in a right anterior oblique projection with caudal angulation (right anterior oblique caudal view) and TFC in the RCA was assessed in a left anterior oblique projection with cranial angulation (left anterior oblique cranial view). All angiograms were filmed at 30 frames/sec.

The TFC, a quantitative method of assessing coronary artery flow, was evaluated on the three main coronary branches (LAD, LCx and RCA), using the protocol described by Gibson \textit{et al.}\textsuperscript{14} Patients with a corrected TFC greater than two standard deviations from the normal published range (36.2 ± 2.6 frames for LAD, 22.2 ± 1.4 for LCx and 20.4 ± 3 for RCA) for the particular vessel were considered as having slow coronary flow, while those whose corrected TFC fell within two standard deviations (cut-off value for the LAD > 38 frames, for the LCx > 28 frames, for the RCA > 26 frames) of the published normal range were labelled as having normal coronary flow.\textsuperscript{14}

Patients with a history of coronary artery disease, recent myocardial infarction or an acute coronary syndrome (within the last two months), coronary vasospasm, coronary ectasia, left ventricular dysfunction, echocardiographically proven left ventricular hypertrophy, uncontrolled hypertension, renal dysfunction and connective tissue disease were excluded from the study. Additionally, patients in both groups who had taken any vitamin supplements within the previous eight weeks were also excluded from the study. All subjects were informed about the study and written consent was obtained from each.

Venous blood samples were collected into tubes containing ethylenediamine tetraacetic acid (EDTA) after an eight-hour fast and immediately stored on ice at 4°C. The plasma was then separated from the cells by centrifugation at 3 000 rpm for 10 min and stored in several aliquots at –80°C until assayed.

**Table 1. Clinical and Laboratory Characteristics of the Groups**

| Variables                  | SCF group (n = 40) | NCF group (n = 40) | p-value |
|----------------------------|-------------------|-------------------|---------|
| Age (year)                 | 51 ± 12           | 48 ± 10           | NS*     |
| Gender: female/male, n (%) | 13 (32.5)/27 (67.5) | 21 (52.5)/19 (47.5) | NS**    |
| Hypertension, n (%)        | 37 (92.5)         | 25 (62.5)         | NS**    |
| Diabetes mellitus, n (%)   | 3 (7.5)           | 2 (5)             | NS**    |
| Smoking, n (%)             | 27 (67.5)         | 12 (30)           | 0.001** |
| Triglycerides (mg/dl)      | 175 ± 112         | 169 ± 103         | NS*     |
| Total cholesterol (mg/dl)  | 187 ± 28          | 179 ± 29          | NS*     |
| HDL cholesterol (mg/dl)    | 38 ± 9            | 42 ± 15           | NS*     |
| LDL cholesterol (mg/dl)    | 107 ± 24          | 104 ± 25          | NS*     |
| CRP (mg/l)                 | 6 ± 3.1           | 5.9 ± 2.6         | NS*     |

Data expressed as mean ± standard deviation.

*Independent samples t-test, **Chi-square test.

**Table 2. Antioxidant Activity and Vitamin E Levels in the Study Groups**

| Variables                  | NCF group (n = 40) | SCF group (n = 40) | p-value* |
|----------------------------|-------------------|-------------------|---------|
| Vitamin E (μmol/l)         | 109.5 ± 31.6      | 85.6 ± 28.5       | 0.001   |
| Antioxidant activity (μmol/l)| 2.5 ± 0.6         | 2.7 ± 0.5         | 0.139   |

*Independent samples t-test.

Chemicals 2-thiobarbituric acid (TBA), α-tocopherol, and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were supplied by Sigma-Aldrich (Steinheim, Germany). All other chemicals used were obtained from Merck Darmstadt (Germany) and were of analytical grade.

Plasma AOA was measured using a method defined as AOA by Koracevic.\textsuperscript{14} In this method, the hydroxyl radical, the most potent biological radical, is produced in a standardised solution of Fe-EDTA complex reacted with hydrogen peroxide by a Fenton-type reaction. These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS).\textsuperscript{14,15} Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. The inhibition of colour development is defined as AOA.

Plasma vitamin E levels were measured using a spectrophotometric method developed by Martinek.\textsuperscript{15} The assay results are expressed in μmol/l.

**Statistical analysis**

Parametric data were expressed as mean ± standard deviation, and categorical data as percentages. SPSS 16.0 (SPSS, Inc, Chicago, Illinois) was used to perform statistical procedures. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. Parametric data were evaluated by independent samples t-test, non-parametric data were evaluated by Mann-Whitney U-test, and categorical data via chi-square test. A p-value ≤ 0.05 was accepted as significant.

**Results**

Clinical and laboratory characteristics of the subjects are summarised in Table 1. Since mean TFC was higher in the study group as it was an enrolment criterion, it is not discussed in detail in the text. There were no significant differences in age, gender, hypertension, lipid profile, CRP levels and diabetes, except for smoking, between patients with SCF and controls. There were no significant differences in AOA levels between the two groups. However, plasma vitamin E levels in the SCF group were significantly lower than in the NCF group (Table 2).

Among the non-smokers in both groups, vitamin E levels were lower in those with SCF compared to the control group (Table 3). However, among smokers in both groups, there was no significant difference in vitamin E levels. There was no significant difference in antioxidant activity between the groups and within each group in smokers and non-smokers with normal coronary flow and slow coronary flow (Table 4).

**Discussion**

Slow coronary flow phenomenon is an important clinical entity because it may be the cause of angina at rest or during exercise,
acute myocardial infarction and hypertension. There have been several hypotheses suggested for slow coronary flow phenomenon since first described in 1972 by Tambe et al. In this theory, endothelial activation and inflammation, which have been reported to be a major contributing factor to many cardiovascular events and demonstrated to be associated with different clinical settings of coronary artery disease, are the most acceptable hypotheses for SCF.

The biological oxidative effects of free radicals on cells, DNA, proteins and lipids are controlled by a spectrum of exogenous proteins and lipids are controlled by a spectrum of exogenous proteins and lipids are controlled by a spectrum of exogenous factors. These phenomena ultimately inhibition of leukocyte adhesion and control of cellular growth phenomenon since first described in 1972 by Tambe et al. In this theory, endothelial activation and inflammation, which have been reported to be a major contributing factor to many cardiovascular events and demonstrated to be associated with different clinical settings of coronary artery disease, are the most acceptable hypotheses for SCF.

TABLE 3. VITAMIN E LEVELS WITHIN GROUPS AND BETWEEN GROUPS

| Variables          | Smokers | Non-smokers | p-value* |
|--------------------|---------|-------------|----------|
|                   | Vitamin E (μmol/l) | Vitamin E (μmol/l) |         |
| NCF group          | 12      | 28          | 0.082    |
| SCF group          | 27      | 13          | 0.015    |
| p-value*           | 0.248   |             |          |

TABLE 4. ANTIOXIDANT ACTIVITY LEVELS WITHIN GROUPS AND BETWEEN GROUPS

| Variables          | Smokers | Non-smokers | p-value* |
|--------------------|---------|-------------|----------|
|                   | Antioxidant activity (μmol/l) | Antioxidant activity (μmol/l) |         |
| NCF group          | 12      | 28          | 0.070    |
| SCF group          | 27      | 13          | 0.938    |
| p-value*           | 0.072   |             |          |

Concluding the lack of significant difference among smokers in both groups, we have no explanation other than the potentially negative influence of smoking on vitamin E levels. Since we did not evaluate the TIMI frame counts individually, it would have been interesting to see how smoking and its frequency could affect coronary flow in smokers, even if they were within the normal range of TIMI frame counts.

Since we did not include TIMI frame counts individually, our study was limited in terms of correlative association between the coronary flow rate and vitamin E levels. This could be investigated in another study. There may also be other confounding factors that were not considered in the current study. Moreover, given the lack of information on the pathophysiology of slow coronary flow, this can be regarded as an initial study.

The negative influence of smoking on vitamin E levels is interesting. Smoking, in addition to its many hazardous effects on the whole body, appears to render the endothelial membrane weak and exposed to the harmful influences of lipophilic radical attacks by having a negative effect on vitamin E levels.

Conclusion

Our study has shown that levels of vitamin E, a membrane protector against oxidative stress, were decreased in patients with slow coronary flow. There also appeared to be a clear negative influence of smoking on vitamin E levels. The association between smoking and vitamin E levels is worth further investigation in larger samples.

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ASSAf recognises top South African scientists

The Academy of Science of South Africa (ASSAf) recognised top South African scientists at its prestigious annual awards ceremony in Pretoria on 23 October 2013. ASSAf annually awards up to two ASSAf Science-for-Society gold medals for outstanding achievement in scientific thinking to the benefit of society. This year, Prof Olive Shisana is among the recipients.

ASSAf prizes reward the scientific fields of health and Earth sciences; and basic sciences, technology and innovation. The award pertains to the scientific fields of health and Earth sciences; and basic sciences, technology and innovation. The AU-TWAS Prize for Young Scientists is managed by the Academy of Science of South Africa (ASSAf), on behalf of its partners, the African Union (AU), The World Academy of Sciences (TWAS) and the South African Department of Science and Technology (DST). Through this award, the AU and TWAS jointly recognise and reward 40, living and working in South Africa, and have a record of research publications in internationally recognised science journals.

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