SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 17: 418-422, 2019

Correlation analysis between 25-hydroxyvitamin D3, vitamin B12 and vitamin C and endothelial function of patients with CHD

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Received January 25, 2018; Accepted October 30, 2018

DOI: 10.3892/etm.2018.6944

Abstract. The aim of the study was to investigate the correlation between levels of 25-hydroxyvitamin D3, vitamin B12 and C and endothelial function of patients with coronary heart disease (CHD). Forty patients with CHD diagnosed in Shanghai Tenth People's Hospital from May 2016 to April 2017 were selected as the observation group. A total of 45 healthy individuals were selected as the control group. The participants included 54 males and 31 females. General information was collected. Peripheral serum biochemical indicators, levels of 25-hydroxyvitamin D3, vitamin B12 and C, homocysteine (Hcy), nitric oxide (NO) and endothelial NOS (eNOS) were measured. Arterial lesions detected by coronary angiography were recorded and indexes of observation and control group were compared for the correlation analysis. A proportion of hypertensive patients and their body mass index (BMI) were significantly higher in the observation than in the control group (P<0.05). Levels of 25-hydroxyvitamin D3, vitamin B12 and C in peripheral blood of observation were significantly lower than those of the control group (P<0.05). Compared with the control group, incidence of single-branch lesion and non-lesion rate were significantly lower, but incidence of double- and triple-branch lesions were significantly higher in observation than in control group (P<0.05). In addition, the level of Hcy in the observation group was higher than that in the control group, but levels of NO and eNOS in observation were significantly lower than those in control group (P<0.05. The correlation analysis revealed that 25-hydroxyvitamin D3 (r=0.792, P<0.01), vitamin B12 (r=0.635, P<0.01) and vitamin C (r=0.703, P<0.01) were negatively correlated with serum NO level. Thus, hypertension, BMI, 25-hydroxyvitamin D3, vitamin B12 and C have independent predictive value for coronary endothelial dysfunction (P<0.05). In conclusion, serum levels of 25-hydroxyvitamin D3, vitamin B12 and C are closely related to vascular endothelial dysfunction in patients with CHD and affect the severity of vascular endothelial dysfunction in patients with CHD.

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Key words: coronary heart disease; 25-hydroxy vitamin D3; vitamin B12; vitamin C

Introduction

The continuous growth of aging population and the changes in genetic and environmental factors and social lifestyles have led to an increase in the incidence of coronary heart disease (CHD) (1). The latest survey data have shown that mortality rate of CHD now is higher than that of malignant tumor, making it a life-threatening disease (2). The continuous improvement of scientific research and clinical practices, have led to improvement of treatment of CHD. However, pathogenesis of this disease remains unclear, and common causes of CHD include hypertension, diabetes and smoking (3).

Correlations between serum 25-hydroxyvitamin D3, vitamin B12 and C levels and CHD have attracted increasing attention (4). Vitamin D is a fat-soluble vitamin that plays an important role in the body to regulate calcium and phosphorus metabolism. The lack of vitamin D in the human body can cause a variety of diseases, such as osteoporosis, and diabetes (5). Vitamin C can prevent the oxidation of lipid cells and eliminate oxygen-free radicals to further prevent CHD (6). Serum homocysteine (Hcy) levels were higher but nitric oxide (NO) levels were lower in CHD patients than in normal controls.

Results showed t hat high Hcy levels can lead to CHD. NO levels are closely related to vascular endothelial injury, and NO level is reduced after vascular endothelial injury (7). Hcy metabolism requires the involvement of vitamin B, C and other substances. Lack of vitamins can lead to hyperhomocysteinemia, thereby causing vascular endothelial dysfunction. Supplementation of vitamins provides a new method for the treatment of vascular endothelial injury in patients with CHD (8).

Materials and methods

Patients. Forty patients with CHD diagnosed in Shanghai Tenth People's Hospital (Shanghai, China) from May 2016

Table I. Comparison of general information between the observation and control groups.

Items	Observation (n=40)	Control (n=45)	P-value
Age (years)	63.46±5.19	62.01±4.48	0.542
Sex (male/female)	25/15	29/16	0.203
Hypertension (cases)	22/40	11/45	0.018
BMI (kg/m^2)	25.77±3.25	21.63±2.98	0.043
Smoking (example)	23/40	20/45	0.061
TC (mmol/l)	5.81±1.04	4.99±1.03	0.325
TG (mmol/l)	1.91±1.06	1.89±0.71	0.783
LDL-C (mmol/l)	3.23±0.90	3.04±0.73	0.621
HDL-C (mmol/l)	1.46±0.49	1.41±0.36	0.719

BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein.

to April 2017 were selected as the observation group. At the same time, 45 healthy individuals with similar age and sex distribution were selected from the physical examination of Shanghai Tenth People's Hospital to serve as the control group. Those participants included 54 males and 31 females, with an age range of 55-78 years, and an average age of 62.3±6.8 years. Based on clinical manifestations, ECG or coronary angiography, the patients showed 70-75% artery stenosis. Exclusion criteria were: i) Patients recently used vitamin B, C or D; ii) with severe hematologic disorders; iii) with severe liver and kidney dysfunction; iv) with cancer; v) with serious infectious diseases; vi) with tuberculosis; and vii) patients without complete clinical data.

The study was approved by the Ethics Committee of Shanghai Tenth People's Hospital. Written informed consent was signed by the patients and/or guardians.

Methods. Clinical data, including age, sex, history of essential hypertension and smoking, weight and height of patients were retrospectively analyzed. All the patients were fasted for 10 h to collect fasting peripheral blood. Serum was collected, and levels of Hcy, NO and endothelial NOS (eNOS) were determined by enzyme-linked immunosorbent assay (ELISA) using kits provided by Beijing Kepu Company (Beijing, China). Solid-phase radioimmunoassay was used to detect vitamin B12 levels. Level of 25-hydroxyvitamin D3 was measured by ELISA. Level of vitamin C was determined by chemical colorimetric method. Other biochemical indicators, including total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) were measured by automatic biochemical analyzer provided by Hitachi.

Diagnostic criteria of coronary artery stenosis: Lesions in one of left coronary artery circumflex, left anterior descending coronary and right coronary artery were diagnosed as single-branch lesions, lesions in two were double-branch lesions and lesions in all three were triple-branch lesions. In addition, left coronary artery lesions, regardless of lesions in other branches, were diagnosed as double-branch lesions. Lesions in both left and right coronary artery were treated as triple-branch lesions.

Statistical methods. SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was used to process the data. Measurement data were expressed as mean ± SD. The Chi-square test was used to compare countable data. Pearson's correlation analysis was used to analyze correlations between two factors. Logistic analysis was performed to explore relevant risk factors. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of general information between observation and control group. There were no significant differences in the age, sex, history of smoking, levels of TC, TG, LDL-C and HDL-C between the observation and control groups (P>0.05). Proportion of hypertensive patients and body mass index (BMI) were significantly higher in the observation than in the control group (P<0.05) (Table I).

Comparison of vitamin levels between the observation and control groups. Serum levels of 25-hydroxyvitamin D3, vitamin B12 and C in observation group were significantly lower than those in control (P<0.05) (Table II).

Comparison of coronary lesions between the observation and control groups. Compared with the control group, incidence of single-branch lesion (14.78%) and non-lesion rate (0%) in observation were significantly lower than those in the control group, but incidences of double- and triple-branch lesions were significantly higher in observation than in control group (P<0.05) (Table III).

Comparison of vascular endothelial function indexes between the observation and control groups. Level of Hcy in peripheral blood of observation was higher than that of control group, but levels of NO and eNOS in the observation were significantly lower than those in control group (P<0.05) (Table IV).

Table II. Comparison of vitamin levels between the observation and control groups.

Items	Observation (n=40)	Control (n=45)	P-values
25-Hydroxyvitamin D3 (pg/ml)	59.80±24.93	91.68±30.67	0.001
Vitamin B12 (pmol/l)	205.33±127.86	436.85±148.92	0.001
Vitamin C (umol/l)	10.85±9.23	37.83±14.29	0.017

Table III. Comparison of coronary lesions between the observation and control groups.

Lesions	Observation (n=40) (%)	Control (n=45) (%)	P-values
Non-lesion	0	54.21	0.001
Single-branch lesion	14.78	37.86	0.024
Double-branch lesion	27.57	6.00	0.001
Triple-branch lesion	57.63	1.93	0.001

Table IV. Comparison of vascular endothelial function indexes between observation and control group.

Endothelial function indexes	Observation (n=40)	Control (n=45)	P-values
Hcy (μmol/l)	29.35±4.67	12.19±2.32	0.001
NO (μmol/l)	42.16±10.38	68.83±12.81	0.014
eNOS (U/ml)	43.86±9.63	68.23±10.47	0.019

Hcy, homocysteine; NO nitric oxide; eNOS, endothelial NOS.

Table V. Logistic regression analysis of the risk factors for vascular endothelial injury in patients with CHD.

Factors	P-value	OR value	95%CI
Age	0.746	0.892	0.103-7.762
Sex	0.109	1.093	0.969-1.158
Hypertension	0.026	6.594	1.927-20.616
BMI	0.005	6.432	1.919-20.143
Smoking	0.054	1.048	0.975-1.227
TC	0.026	1.424	0.576-3.573
TG	0.527	1.458	0.466-4.493
LDL-c	0.052	2.743	0.965-8.137
HDL-c	0.951	0.078	0.443-20.713
25-hydroxyvitamin D3	0.001	1.004	0.979-1.033
Vitamin B12	0.022	1.057	0.959-1.118
Vitamin C	0.019	1.034	0.984-1.035

BMI, body mass index; TC, total cholesterol, TG, triglyceride; LDL-c, low density lipoprotein; HDL-c, high density lipoprotein.

Correlation analysis of serum levels of 25-hydroxyvitamin D3, vitamin B12 and C, and NO. Correlation analysis of 25-hydroxyvitamin D3, vitamin B12 and C and serum NO levels showed that 25-hydroxyvitamin D3 (r=0.792, P<0.01), vitamin B12 (r=0.635, P<0.01) and vitamin C (r=0.703, P<0.01) were positively correlated with serum NO level. (Figs. 1-3) for details.

Logistic regression analysis of the risk factors for vascular endothelial injury in patients with CHD. Hypertension, BMI, 25-hydroxyvitamin D3, vitamin B12 and C have independent predictive value for coronary endothelial dysfunction (P<0.05). (Table V).

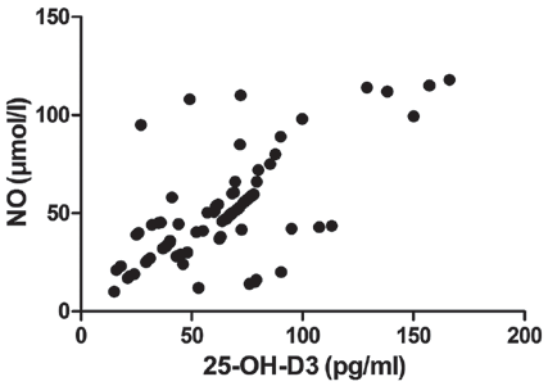

Figure 1. Correlation between levels of 25-hydroxyvitamin D3 and NO levels. NO nitric oxide.

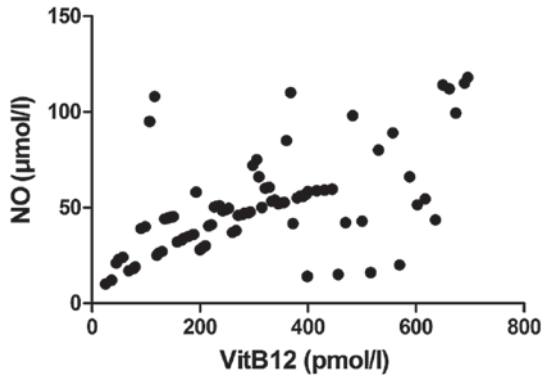

Figure 2. Correlation between levels of vitamin B12 and NO levels. NO nitric oxide.

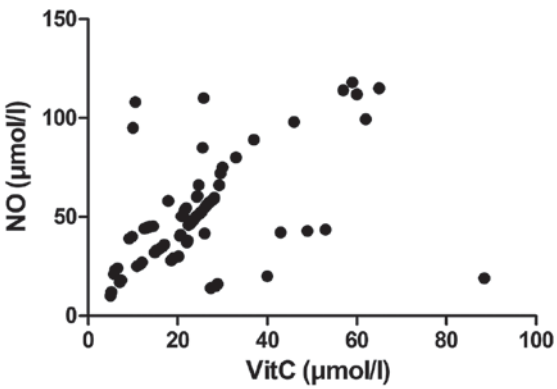

Figure 3. Correlation between levels of vitamin C and NO levels. NO nitric oxide.

Discussion

The main pathogenesis of coronary atherosclerotic heart disease is atherosclerosis caused by varying degrees of stenosis and damage, which results in increased blood flow resistance. Under this condition, oxygen and blood supply to myocardial cells is reduced, leading to necrosis or even death (9). Advances in clinical research have shown that

Hcy, NO and other substances can reflect impaired coronary vascular endothelial function (10). Hcy is an intermediate product of the metabolism of methionine and cysteine and cannot be synthesized in human body. Hcy has to be formed after methylation of methionine in food by ingesting food (11). After demethylation, methionine in the food is converted to Hcy (11). Therefore, abnormal metabolic process may lead to the accumulation of Hcy, and excessive Hcy can also enter blood circulation (12) to damage vascular endothelial cells, resulting in the excessive growth of vascular smooth muscle cells and over-reaction of the inflammatory system, thereby obstructing lipid metabolism, and inducing vascular endothelial dysfunction and atherosclerosis (13). Vascular endothelial cells, on the other hand, can also synthesize and release NO by catalyzing the conversion of L-arginine to NO via eNOS (14). Thus, with the inhibitory effects on atherosclerosis, NO levels can reflect endothelial cell function and low NO level indicates impaired vascular endothelial function (15).

Vitamins play critical roles in the human body, for example, vitamin D can be synthesized with the involvement of sunlight and play its functions through 25-hydroxy vitamin D3 receptors (16). Vitamin D receptor generally exists in vascular smooth muscle cells and intestinal mucosal cells. Low vitamin D content can cause pathological diseases, such as diabetes and osteoporosis (17). An increasing number of studies have shown that low levels of vitamin D can cause increased incidence and mortality of cardiovascular diseases (18). This study found that 25-hydroxyvitamin D3 level was significantly lower in patients with CHD than in healthy individuals, and 25-hydroxyvitamin D3 and NO levels were positively correlated with each other. Hcy synthesis in the human body requires the involvement of coenzyme such as vitamin B12, and low vitamin B12 levels can lead to homocysteinemia (19). Studies have shown that vitamin B12 is an independent risk factor for CHD, and increasing the level of vitamin B12 can reduce Hcy level and relieve the impaired vascular endothelial function (19). In this study, vitamin B12 levels were significantly lower in patients with CHD than in control group, and vitamin B12 levels were positively correlated with NO levels. In addition, other vitamins, such as vitamin C, an important antioxidant, can effectively prevent CHD and protect vascular endothelial function by removing oxygen free radicals and decreasing peroxidation of serum lipids (20). Therefore, supplementation of vitamin has important clinical value in preventing CHD and improving prognosis of vascular endothelial dysfunction.

Acknowledgements

Not applicable.

Funding

This study was supported by the Natural Science Foundation of China (NSFC), grants nos. 81270193 and 30800466 (Y.-D. W.).

Availability of data and materials

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

Authors' contributions

XQ was a major contributor in the design of the study. LQ participated in the writing of the manuscript. JL participated in the analysis and discussion of the data. LQ and BL were mainly responsible for collecting the general data of the patients. JZ and YW carried out additional analysis of patient data. HL was a major contributor in designing the methods and interpreting the results. YW was responsible for revising and finalizing this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai Tenth People's Hospital, Tenth People's Hospital of Tongji University (Shanghai, China). Signed written informed consents were obtained from the patients and/or guardians.

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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