# Serum levels of homocysteine and circulating antioxidants associated with heart rate variability in patients with unstable angina pectoris

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*To the Editor*: Autonomic dysfunction is an important factor in the pathogenesis of unstable angina pectoris (UAP). It affects coronary artery systolic function, induces coronary spasm, aggravates myocardial ischemia, reduces ventricular fibrillation threshold, and increases the incidence of sudden cardiac death in UAP.[1] Oxidative stress, which is defined as an excessive production of reactive oxygenated species (ROS) that cannot be counteracted by the action of antioxidants, has been proposed as an underlying mechanism that contributes to the initiation and progression of cardiovascular diseases.[2] Although the cause-and-effect relationship between autonomic dysfunction and oxidative stress has not been determined, it has been suggested that oxidative stress is one of the most crucial mechanisms that contributes to the onset and progression of neurodegenerative diseases by causing excitotoxicity, neuronal loss, axonal impairment.[3] Studies have reported that serum levels of homocysteine (Hcy), uric acid, albumin, and bilirubin are simple laboratory parameters that reflect natural oxidative-antioxidant status. Furthermore, previous study showed that increased oxidative stress predicted autonomic dysfunction in diabetes. Heart rate variability (HRV) has considerable potential to evaluate the role of autonomic nervous system fluctuations in healthy individuals and in patients with various cardiovascular disorders.[4] It is reported that suppressed HRV was associated with increased oxidative stress in both essential hypertensive and prehypertensive patients.[5] Nevertheless, whether HRV is associated with endogenous oxidative-antioxidant substances in circulation has not been investigated. Therefore, we performed a cross-sectional study to investigate the correlation between HRV and circulating natural oxidative-antioxidant substances in patients with unstable angina.

Two hundred sixteen consecutive Chinese patients who were admitted in Affiliated Hospital of Shandong University of Traditional Chinese Medicine Cardiovascular department within 48 h for unstable angina between February 1, 2015 and May 1, 2017 were included in our study. Patients were diagnosed with unstable angina if they presented at least one of the 3 following features: (1) angina occurring at rest and prolonged, greater than 20 minutes; (2) new onset angina of at least Canadian Cardiovascular Society class III severity (within 1 month); and (3) previously diagnosed angina that had become distinctly more frequent, longer in duration, or lower in threshold: any of these findings should be associated with the absence of increased markers of myocardial necrosis (creatine kinase-MB isoenzyme and troponin I), with or without ST-segment or T-wave abnormalities on an electrocardiogram (ECG).[6] Patients were not eligible if they presented with one of the following conditions: hemodynamically unstable valvular heart disease, congenital heart diseases, recent history of acute myocardial infarction or percutaneous transluminal coronary angioplasty (<4 weeks prior to hospitalization), persistent atrial fibrillation and severe heart conduction disorders, acute heart failure or acute attack of chronic heart failure, abnormal hepatic function (Aspartate aminotransferase [AST] and Alanine aminotransferase [ALT] >3 times the upper normal limit), renal failure (serum creatinine >1.5 mg/dL), recent gout, severe respiratory illness, malignant tumor, connective tissue diseases, recent surgery, and recent infectious and inflammatory disease. Finally, 190 patients with unstable angina but without typical changes in the ECG and troponin were enrolled.

The following data of those eligible patients were collected from their medical records: (1) Demographic properties, (2) Biochemical measures, (3) HRV. Analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, IL, USA). For study purposes, differences in characteristics between participants with standard deviation of all NN intervals (SDNN) < 100 ms (suppressed HRV) and those with SDNN ≥ 100 ms (normal

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HRV) were compared using Student's *t*-test for continuous variables and a chi-square test for dichotomous variables. Data are expressed as the mean ± standard deviation (SD) for normal distributed variables and frequencies and percentages (*n* [%]) for categorical variables. Univariate correlation analysis was carried out using the Pearson or Spearman correlation test. Stepwise multivariate linear regression analysis (inclusion at 0.05 and exclusion at 0.01) was used to identify the association between HRV parameters and serum levels of Hcy and circulating antioxidants. Bonferroni correction was further conducted to control for the effect of multiple testing ($P < 0.008$). Binary logistic regression analysis was performed to identify significant independent factors related to suppressed HRV. Clinical variables (age, sex, smoking, body weight, and alcohol drinking), history of diseases (hypertension, diabetes, chronic heart failure, and stroke), heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP), medical therapy (diuretics, β-blockers, angiotensin-converting enzyme inhibitor [ACEI]/Angiotensin receptor blocker [ARB], statins, and insulin), biochemical markers (albumin, bilirubin, urea acid, Hcy, creatinine, blood fasting sugar, triglycerides, total cholesterol, and HDL-C), and hemoglobin A1c were regarded as independent variables. The distributions of total power (TP), low frequency power (LF), and high frequency power (HF) were skewed and not normally distributed, and they were converted to natural logarithms before statistical analyses were performed. The level of significance was set at the 2-sided $P < 0.05$ level.

Table 1 compares the clinical characteristics of 94 patients with suppressed HRV and 96 patients with normal HRV

**Table 1: Clinical characteristics of the unstable angina pectoris patients according to SDNN**

| Characteristics | SDNN < 100 ms (*n* = 94) | SDNN ≥ 100 ms (*n* = 96) | *P* |
|---|---|---|---|
| Sex (male/female), *n* | 43/51 | 32/64 | 0.102 |
| Age (years) | 69.35 ± 10.23 | 64.67 ± 11.26 | 0.003 |
| Body weight (kg) | 66.76 ± 10.24 | 66.70 ± 11.08 | 0.972 |
| Heart rate (beats/min) | 73.59 ± 15.88 | 67.40 ± 10.84 | 0.002 |
| Smoking, *n* (%) | 39 (41.5) | 30 (31.2) | 0.175 |
| Alcohol intake, *n* (%) | 42 (44.8) | 29 (30.2) | 0.051 |
| SBP (mmHg) | 136.69 ± 20.52 | 135.50 ± 24.09 | 0.716 |
| DBP (mmHg) | 78.52 ± 13.48 | 80.32 ± 11.61 | 0.324 |
| Glucose (mmol/L) | 6.46 ± 2.47 | 5.93 ± 2.17 | 0.124 |
| Triglycerides (mmol/L) | 1.72 ± 1.08 | 1.49 ± 1.15 | 0.161 |
| Total cholesterol (mmol/L) | 4.71 ± 1.12 | 4.85 ± 1.18 | 0.422 |
| HDL-C (mmol/L) | 1.06 ± 0.23 | 1.23 ± 0.70 | 0.026 |
| LDL-C (mmol/L) | 2.79 ± 0.98 | 2.89 ± 0.90 | 0.445 |
| Hemoglobin A1c (%) | 5.89 ± 0.65 | 5.62 ± 0.70 | 0.009 |
| Creatine kinase (U/L) | 78.37 ± 23.73 | 81.51 ± 30.34 | 0.103 |
| Creatine kinase-MB isoenzyme (U/L) | 8.71 ± 2.31 | 8.35 ± 3.53 | 0.305 |
| Troponin I (ng/mL) | 0.16 ± 0.05 | 0.22 ± 0.08 | 0.223 |
| ALT (U/L) | 21.27 ± 13.70 | 22.92 ± 12.07 | 0.386 |
| AST (U/L) | 17.77 ± 10.86 | 20.34 ± 14.70 | 0.179 |
| Creatinine (μmol/L) | 67.83 ± 25.11 | 60.34 ± 14.52 | 0.014 |
| Urea nitrogen (mmol/L) | 5.50 ± 2.18 | 4.86 ± 1.59 | 0.025 |
| Albumin (g/L) | 38.47 ± 3.80 | 40.72 ± 3.26 | <0.001 |
| Uric acid (μmol/L) | 5.50 ± 2.18 | 4.86 ± 1.59 | 0.006 |
| Homocysteine (μmol/L) | 15.93 ± 7.00 | 12.66 ± 4.95 | <0.001 |
| Total bilirubin (μmol/L) | 13.49 ± 6.30 | 13.36 ± 4.76 | 0.881 |
| Direct bilirubin (μmol/L) | 2.38 ± 1.00 | 2.85 ± 1.36 | 0.009 |
| indirect bilirubin (μmol/L) | 10.78 ± 4.86 | 10.69 ± 3.62 | 0.891 |
| History of diseases, *n* (%) | | | |
| Hypertension | 68 (72.3) | 58 (60.4) | 0.082 |
| Diabetes | 30 (31.9) | 19 (19.8) | 0.068 |
| Chronic heart failure | 20 (21.3) | 6 (6.3) | 0.003 |
| Stroke | 25 (26.6) | 19 (19.8) | 0.174 |
| Drugs, *n* (%) | | | |
| Beta-blockers | 72 (76.6) | 66 (68.8) | 0.256 |
| Calcium antagonists | 36 (38.3) | 27 (28.1) | 0.166 |
| Diuretics | 7 (7.4) | 2 (2.1) | 0.080 |
| ACE-inhibitors | 57 (60.6) | 48 (50.0) | 0.148 |
| Statins | 82 (87.2) | 74 (77.1) | 0.088 |
| Insulin | 6 (6.4) | 4 (4.2) | 0.534 |

Data are mean ± SD for continuous variables. For frequency data, chi square-test was applied. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ACE: Angiotensin converting enzyme.

as per a cutoff of 100 ms for SDNN. Patients with suppressed HRV were statistically significantly older ($P=0.003$), and their heart rate was statistically significantly higher compared with patients with normal HRV ($P=0.002$). Relative to patients with normal HRV, those with suppressed HRV had statistically significantly lower levels of high-density lipoprotein cholesterol (HDL-C), albumin, and direct bilirubin and higher levels of hemoglobin A1c, serum creatinine, urea nitrogen, uric acid, and Hcy ($P<0.05$), and they were also more likely to have chronic heart failure ($P=0.003$).

Univariate correlations of serum Hcy, albumin, uric acid, and bilirubin with HRV parameters after Bonferroni correction are shown in Table 2. The time-domain HRV parameter SDNN value was positively correlated with serum levels of albumin ($r=0.296$, $P<0.001$) and negatively correlated with levels of Hcy ($r=-0.302$, $P<0.001$). Square root of the mean of the sum of the squares of differences between adjacent NN interval (RMSSD) was positively correlated with serum concentrations of albumin ($r=0.212$, $P=0.004$) and direct bilirubin ($r=0.292$, $P<0.001$). Additionally, the frequency-domain HRV parameter natural logarithms low frequency power (Ln LF) was positively correlated with serum levels of albumin ($r=0.201$, $P=0.007$) and direct bilirubin ($r=0.209$, $P=0.006$).

After adjusting for possible confounding by clinical variables (age, sex, smoking, body weight, and alcohol drinking), history of diseases (hypertension, diabetes, chronic heart failure, and stroke), heart rate, SBP and DBP, medical therapy (diuretics, β-blockers, ACEI/ARB, statins, and insulin), biochemical markers (creatinine, blood fasting sugar, triglycerides, total cholesterol, and HDL-C), and hemoglobin A1c, multiple linear regression analyses and logistic regression analysis were performed. SDNN remained positively correlated with albumin levels (beta = 0.393, $P<0.001$) and inversely correlated with Hcy levels (beta = −0.316, $P<0.001$). Moreover, SDNN was associated with hemoglobin A1c (beta = −0.268, $P<0.001$), SBP (beta = −0.183, $P=0.005$) and chronic heart failure (beta = −0.144, $P=0.03$). Additionally, RMSSD was positively correlated with HDL-C levels (beta = 0.268, $P<0.001$), direct bilirubin (beta = 0.242, $P=0.001$), albumin (beta = 0.242, $P=0.001$) and age (beta = 0.256, $P=0.001$), while it was inversely correlated with history of hypertension (beta = −0.176, $P=0.013$). There was no correlation between frequency-domain parameters and levels of Hcy, albumin or bilirubin.

Binary logistic regression analysis revealed that decreased albumin levels (OR = 0.740 [0.656–0.835], $P<0.001$) and increased Hcy levels (OR = 1.188 [1.091–1.294], $P<0.001$) and hemoglobin A1c (OR = 2.067 [1.149, 3.721], $P=0.015$) were independent variables predicting the occurrence of suppressed HRV.

Hcy is the demethylated metabolite of the essential amino acid methionine. The increased blood levels of Hcy can aggravate neurotoxicity by causing oxidative stress.[7] At present, there are very few studies that provide evidence supporting the association between elevated Hcy levels and autonomic neuropathy. In our present study, we found that increased Hcy was inversely correlated with SDNN, indicating that increased Hcy levels could be an indicator of depressed HRV in patients with unstable angina. We believed that this relationship might be explained by the direct neurotoxicity of Hcy and the insufficient supply of blood indirectly caused by Hcy-induced atherosclerosis.

Albumin is the main extracellular molecule responsible for maintaining the plasma redox state. It exerts antioxidant activity by limiting ROS production and by scavenging ROS.[8] In patients with type 2 diabetes, serum albumin was significantly associated with the severity of neuropathy reflected by median motor nerve conduction velocity after adjusting for confounding variables.[9] In our study, we found that decreased albumin levels were inversely correlated with SDNN and RMSSD values after adjusting for confounding variables; moreover, decreased albumin could indicate depressed HRV in patients with unstable angina. This finding indicated that decreased albumin levels might reflect autonomic dysfunction in unstable angina patients.

Bilirubin, the end product of heme catabolism, is convincingly regarded as an endogenous antioxidant and antiinflammatory molecule. Studies have consistently shown that increased bilirubin levels were negatively correlated with the occurrence of cardiovascular diseases, hypertension, diabetes, and metabolic syndrome.[10] Moreover, in type 2 diabetic patients, physiological serum bilirubin concentrations are inversely associated with the

**Table 2: Pearson or Spearman correlation analysis between HRV parameters and serum levels of albumin, uric acid, Hcy, and bilirubin after Bonferroni correction**

| | Albumin | | Uric acid | | Homocysteine | | Total bilirubin | | Indirect bilirubin | | Direct bilirubin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Items | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| SDNN | 0.296 | <0.001 | −0.155 | 0.039 | −0.302 | <0.001 | 0.105 | 0.163 | 0.014 | 0.852 | 0.173 | 0.022 |
| RMSSD | 0.212 | 0.004 | −0.027 | 0.716 | 0.020 | 0.788 | 0.139 | 0.060 | 0.147 | 0.048 | 0.292 | <0.001 |
| Ln LF* | 0.201 | 0.007 | 0.062 | 0.417 | −0.084 | 0.271 | 0.172 | 0.023 | 0.085 | 0.262 | 0.209 | 0.006 |
| Ln HF* | 0.038 | 0.610 | −0.050 | 0.510 | −0.004 | 0.961 | 0.072 | 0.345 | 0.065 | 0.389 | 0.136 | 0.073 |
| Ln TP* | 0.199 | 0.009 | −0.041 | 0.592 | −0.138 | 0.075 | 0.064 | 0.408 | −0.006 | 0.939 | 0.134 | 0.083 |
| LF/HF | 0.097 | 0.196 | 0.099 | 0.191 | 0.013 | 0.870 | 0.148 | 0.051 | 0.086 | 0.253 | 0.112 | 0.141 |

* Values of LF, HF and TP were converted to natural logarithms. SDNN: Standard deviation of all NN intervals; RMSSD: Square root of the mean of the sum of the squares of differences between adjacent NN interval; Ln LF: natural logarithms Low frequency; Ln HF: natural logarithms High frequency; Ln TP: natural logarithms total power.

prevalence of cardiovascular autonomic neuropathy.[11] In agreement with these studies, our results show that blood levels of direct bilirubin were positively correlated with RMSSD, indicating that unstable angina patients with higher levels of direct bilirubin have better parasympathetic activity. Nevertheless, this result did not mean that higher bilirubin levels are completely beneficial to the autonomic nervous system because excessively high levels of bilirubin can be neurotoxic.[12]

In conclusion, our study indicates that in patients with unstable angina, the blood levels of albumin, direct bilirubin, and Hcy are associated with HRV. These commonly used laboratory parameters together with depressed HRV could be useful in the diagnosis of cardiac autonomic neuropathy.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There is no conflicts of interest.

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