Serum adenosine deaminase activity and coronary artery disease: a retrospective case-control study based on 9929 participants

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Abstract
Background: Adenosine deaminase (ADA) regulates purine metabolism through the conversion of adenosine to uric acid (UA). Adenosine and UA are closely associated with cardiovascular events, but the correlation between serum ADA activity and coronary artery disease (CAD) has not been defined.
Methods: We performed a hospital-based retrospective case-control study that included a total of 5212 patients with CAD and 4717 sex- and age-matched controls. The serum activity of ADA was determined by peroxidase assays in an automatic biochemistry analyzer.
Results: Serum ADA activity in the CAD group (10.08 ± 3.57 U/l) was significantly lower than that of the control group (11.71 ± 4.20 U/l, p < 0.001). After adjusting for conventional factors, serum ADA activity negatively correlated with the presence of CAD (odds ratio = 0.852, 95% confidence interval: 0.839–0.865, p < 0.001). Among the patients with CAD, serum ADA activity was lowest in patients with myocardial infarction (MI: 9.77 ± 3.80 U/l). Diabetes mellitus and hypertension increased the serum ADA activity in CAD patients.
Conclusions: Serum ADA activity is significantly attenuated in patients with CAD, particularly in MI. We propose a mechanism by which the body maintains adenosine levels to protect the cardiovascular system in the event of CAD.

Keywords: adenosine, adenosine deaminase, coronary artery disease

Introduction
Adenosine deaminase (ADA) catalyzes the deamination of adenosine to inosine and is a key enzyme in purine catabolism. As a metabolic enzyme, ADA is ubiquitously expressed in various cells/tissues, including the lymphatic system. ADA is necessary for the proliferation and differentiation of T lymphocytes, and the maturation and function of monocytes and macrophages. ADA deficiency leads to cellular and humoral immunodeficiency, which manifests as severe combined immunodeficiency disease.

Serum ADA activity is used to evaluate diseases related to cell-mediated immune responses, and is considered a useful tool in the monitoring of clinical status. As a nonspecific indicator of cellular immunity, altered ADA activity has been detected in many diseases, including tuberculosis, rheumatoid arthritis, systemic lupus erythematosus, and liver diseases.

The metabolism of adenosine, homocysteine (Hcy), and uric acid (UA) are biochemically interrelated. S-adenosyl-homocysteine hydrolase catalyzes the reversible hydrolysis of S-adenosyl-homocysteine (SAH) to Hcy and adenosine in the liver. Adenosine is a surrogate indicator of Hcy. UA is the end product of adenosine metabolism, and Hcy and UA cause endothelial dysfunction.
and are widely recognized risk factors for cardiovascular disease.\textsuperscript{10,11} As a protector of the cardiovascular system, adenosine induces vasodilation, regulates the activity of the sympathetic nervous system, prevents thrombosis, regulates blood pressure and heart rate, and has increased activity in the serum of patients with coronary artery disease (CAD).\textsuperscript{12} Since ADA catalyzes the irreversible deamination of adenosine, its relationship to cardiovascular disease remains a concern, particularly in animal experiments and studies assessing the relationship between ADA gene variants and the risk of CAD.\textsuperscript{13,14} However, studies investigating the correlation between ADA activity and the occurrence of CAD in large sample sizes are sparse. In this study, we explored this relationship through a retrospective case-control study.

Materials and methods

Subjects

In this hospital-based retrospective case-control study, all participants visited The Affiliated Hospital of Qingdao University between December 2012 and June 2018. A total of 5212 patients who met the CAD diagnostic criteria were enrolled in the study upon the onset of symptoms and were hospitalized for coronary angiography. The diagnosis and severity of CAD were assessed by a cardiologist who used angiographic findings. Patients with autoimmune disease, liver disease, tuberculosis, tumors, and other serious illnesses that interfered with the results of the study were excluded. The 4717 controls were age and sex matched and showed no signs or symptoms of cardiovascular events. Verbal informed consent was obtained from all participants upon description of the study protocol. The Ethics Committee of our hospital approved the study (approval number: 20190008), and the protocol was confirmed using the ethical guidelines of the Helsinki declaration of 1975.

Clinical parameters

Data on physical examinations, including smoking and drinking habits, sex, age, body mass index (BMI), hypertension, diabetes mellitus (DM), and medication [angiotensin-converting-enzyme inhibitors (ACEIs)/angiotensin-receptor blocker, \(\beta\)-blocker, statin] history were recorded. Coronary angiography was used to identify the number of diseased vessels in the patients. Four major coronary artery branches (left main, left anterior descending, left circumflex, and right coronary artery) were evaluated and a luminal stenosis degree of 50\% or more was defined as a significant lesion. Patients were defined as having single, double, or triple branch involvement if they had one, two, or three or more branches involved, respectively.

Biochemical measurements

Whole blood was collected by vacuum blood collection without anticoagulants, and was centrifuged at 1500g for 15 min. The participants fasted for at least 8–10 h, and blood was collected in the morning. Serum activity/concentrations of alanine aminotransferase (ALT), serum creatinine (SCr), low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), fasting blood glucose (FBG), UA, and ADA were determined using an automatic biochemistry analyzer (Hitachi HCP-7600, Hitachi, Japan).

ADA activity was determined by peroxidase assays. ADA catalyzes adenosine deamination to inosine. Purine nucleoside phosphorylase catalyzes the conversion of inosine into hypoxanthine. Hypoxanthine is oxidized by xanthine oxidase to UA and hydrogen peroxide (\(\text{H}_2\text{O}_2\)). \(\text{H}_2\text{O}_2\) further reacts with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase to generate quinone, the kinetics of which can be monitored. One unit of ADA is defined as the amount of ADA that generates 1 µmol/l/min of inosine from adenosine at 37\(^\circ\)C. The enzymatic reaction scheme is shown below:

\[
\begin{align*}
(1) \text{Adenosine} + \text{H}_2\text{O} & \xrightarrow{\text{ADA}} \text{Inosine} + \text{NH}_3 \\
(2) \text{Inosine} + \text{Pi} & \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose-1-phosphate} \\
(3) \text{Hypoxanthine} + 2\text{H}_2\text{O} & \xrightarrow{\text{XOD}} \text{Uric acid} + 2\text{H}_2\text{O}_2 \\
(4) 2\text{H}_2\text{O}_2 + 4\text{AA} + \text{EHSPT} & \xrightarrow{\text{POD}} 4\text{H}_2\text{O} + \text{Quinone dye} (\lambda = 556 \text{nm})
\end{align*}
\]

Statistical analysis

All data were analyzed with SPSS statistical software (version 13.0; SPSS Inc., Chicago, Illinois, USA). Values represent the mean ± standard deviation (SD) if not otherwise specified.
distribution of categorical variables was expressed as frequencies and percentages and comparisons were calculated using the chi-square test or Fisher’s exact test, as appropriate. Comparisons between groups for study variables were performed using an unpaired student’s $t$ test or one-way analysis of variance (ANOVA) for normally distributed parameters. Logistic regression was used to test the interactive effects of other variables on the observed association between serum ADA activity and CAD. All statistical tests were two sided, and $p < 0.05$ was recognized as statistically significant.

**Results**

A total of 5212 CAD patients (mean age 61.66 ± 9.86; 65.74% men) and 4717 controls (mean age 61.82 ± 11.78; 64.55% men) were enrolled. No significant differences were observed between CAD patients and controls regarding sex, age, and SCr. However, BMI, FBG, TG, LDL-C, and ALT activity/levels were significantly elevated in CAD patients. In addition, the patient group had higher rates of hypertension, DM, smoking and drinking rates compared with controls. In the CAD patient group, 1873 patients were diagnosed with MI. CAD patients included 1979 patients with single-diseased vessels, 1274 patients with double-diseased vessels, and 1083 patients with triple-diseased vessels. The clinical characteristics of all participants are summarized in Table 1.

Pearson’s correlation analysis revealed that serum ADA activity positively correlated with age.

**Table 1.** Demographic and clinical characteristics of CAD patients and controls.

| Variable     | CAD ($n = 5212$) | Control ($n = 4717$) | $p$ value |
|--------------|-----------------|---------------------|-----------|
| Sex, male n [%]# | 3427 [65.74] | 3045 [64.55] | 0.218 |
| Age, years* | 61.66 ± 9.86 | 61.82 ± 11.78 | 0.465 |
| BMI [kg/m²]* | 25.58 ± 3.35 | 24.85 ± 3.34 | <0.05 |
| Hypertension, n [%]# | 3313 [63.56] | 1155 [24.49] | <0.05 |
| Diabetes, n [%]# | 1420 [27.24] | 663 [14.06] | <0.05 |
| Smoking, n [%]# | 2431 [46.64] | 1117 [23.68] | <0.05 |
| Drinking, n [%]# | 1779 [34.13] | 955 [20.25] | <0.05 |
| FBG, mmol/l* | 6.08 ± 2.25 | 5.63 ± 1.71 | <0.05 |
| TG, mmol/l* | 1.75 ± 1.51 | 1.54 ± 1.32 | <0.05 |
| TC, mmol/l* | 4.56 ± 1.18 | 3.98 ± 1.13 | <0.05 |
| UA, μmol/l* | 316.68 ± 83.85 | 306.97 ± 82.96 | <0.05 |
| HDL-C, mmol/l* | 2.19 ± 1.46 | 2.57 ± 1.45 | <0.05 |
| LDL-C, mmol/l* | 2.75 ± 0.96 | 2.39 ± 1.00 | <0.05 |
| SCr, μmol/l* | 82.82 ± 17.58 | 82.98 ± 14.35 | 0.618 |
| ALT, U/l* | 23.58 ± 10.41 | 20.30 ± 9.73 | <0.05 |
| Medications | – | – | – |

(Continued)
and FBG ($r = 0.237, p < 0.001$) in CAD patients. In addition, a negative relationship in UA ($r = -0.057, p < 0.001$) and SCr ($r = -0.097, p < 0.001$) were observed. DM, hypertension, and drinking and smoking status significantly influenced serum ADA activity in patients with CAD. DM and hypertension significantly increased serum ADA activity in CAD patients, while smoking and drinking had the opposite effect. These results are listed in Table 2.

In this retrospective study, serum ADA activity was determined in all participants, and was closely related to the presence of CAD. In CAD patients, the mean activity of serum ADA was $10.08 \pm 3.57$ U/l. Serum ADA activity was significantly attenuated in CAD patients compared with controls ($11.71 \pm 4.20$ U/l, unpaired $t$ test, $p < 0.001$; Figure 1). After further adjustment for BMI, FBG, TG, TC, HDL-C, LDL-C, UA, ALT, smoking, drinking, hypertension, DM status and medications, serum ADA activity was significantly associated with the presence of CAD [odds ratio (OR) = 0.852, 95% confidence interval: 0.839–0.865, $p < 0.001$]. The association results were similar in OR adjustment models, which included different conventional factors. The main results are listed in Table 3.

The serum ADA activity in CAD patients with stable angina, unstable angina, and MI were $10.08 \pm 3.57$ U/l ($n = 5212$), $11.71 \pm 4.20$ U/l ($n = 4717$), and $9.77 \pm 3.80$ U/l ($n = 1873$), respectively. The serum ADA activity in patients with MI was significantly attenuated compared with patients with stable and unstable angina (one-way ANOVA, $p < 0.001$; Figure 2). No correlation between the number of diseased vessels and serum ADA activity was observed in CAD patients.

### Table 1. (Continued)

| Variable                           | CAD ($n = 5212$) | Control ($n = 4717$) | $p$ value |
|------------------------------------|------------------|----------------------|-----------|
| ACEls/ARP, n (%)#                  | 1996 (38.30)     | 492 (10.43)          | <0.05     |
| β-blocker, n (%)#                  | 3231 (61.99)     | 1550 (32.86)         | <0.05     |
| Statin, n [%]#                     | 2818 (54.07)     | 785 (16.64)          | <0.05     |
| Myocardial infarction, n [%]       | 1873 (35.94)     | –                    | –         |
| Stable angina, n [%]               | 1332 (25.55)     | –                    | –         |
| Unstable angina, n [%]             | 2007 (38.51)     | –                    | –         |
| Severity of CAD                    | –                | –                    | –         |
| Single-diseased vessels, n [%]     | 1979 (37.97)     | –                    | –         |
| Double-diseased vessels, n [%]     | 1274 (24.44)     | –                    | –         |
| Triple-diseased vessels, n [%]     | 1083 (20.78)     | –                    | –         |
| ADA, U/l*                          | $10.08 \pm 3.57$ | $11.71 \pm 4.20$     | <0.05     |
| Male, U/l*                         | $9.40 \pm 3.24$  | $11.01 \pm 4.05$     | <0.05     |
| Female, U/l*                       | $11.39 \pm 3.80$ | $12.99 \pm 4.18$     | <0.05     |

*Categorical variables are expressed as percentages. $p$ values of the categorical variables were calculated by $\chi^2$ test.

*Continuous variables are expressed as the mean ± SD. $p$ values of the continuous variables were calculated using unpaired $t$ test.

ACEls/ARP, angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker; ADA, adenosine deaminase; ALT, alanine aminotransferase; BMI, body mass index; CAD, coronary artery disease; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SCr, Serum creatinine; SD, standard deviation; TC, total cholesterol; TG, triglyceride; UA, uric acid.
Table 2. Clinical parameters and ADA activity.

|                 | CAD patients with | Nonhypertension | Hypertension | Nondiabetes | Diabetes |
|-----------------|------------------|-----------------|--------------|-------------|----------|
| Patients Number | 1420             | 3313            | 3792         | 1899        | 2431     |
| ADA activity    | 11.27 ± 4.04     | 10.18 ± 3.61    | 9.63 ± 3.27  | 10.30 ± 3.28| 9.30 ± 3.70|
| p               | <0.001           | 0.01            | <0.001       | 0.01        | <0.001   |

ADA is expressed as the mean ± SD. ADA, adenosine deaminase; CAD, coronary artery disease; SD, standard deviation.

Figure 1. Serum ADA activity in patient and control groups. The mean activity of serum ADA in CAD patients was 10.08 ± 3.57 U/L (n = 5212). Serum ADA activity was significantly attenuated in controls (11.71 ± 4.20 U/L, unpaired t test, n = 4717, p < 0.000). ADA, adenosine deaminase; CAD, coronary artery disease.

Discussion

This study was the first to show that low levels of serum ADA activity independently correlates with CAD occurrence. In addition, serum ADA activity was significantly attenuated in CAD patients with MI, compared with those with stable and unstable angina pectoris.

A variety of metabolites related to cardiovascular disease are generated in the methionine cycle and during one carbon metabolism (Figure 3). These include Hcy, asymmetric dimethylarginine (ADMA), and UA. These metabolites act on the endothelium of coronary arteries leading to endothelial dysfunction and cardiovascular disease through peroxidation injury, reduced nitric oxide production, and bioavailability. In addition, various enzymes, cofactors and substrates involved in this pathway are closely related to the risk of cardiovascular disease, including folate, vitamin B<sub>12</sub>, L-arginine, and methylene tetrahydrofolate reductase (MTHFR). In our previous studies, we demonstrated that serum UA and ADMA concentrations were associated with the presence and severity of CAD, revealing the mechanisms of ADMA on endothelial dysfunction in human internal mammary arteries and porcine coronary arteries. In addition, the association between MTHFR gene variants and the risk of MI was identified in our previous meta-analysis.

In the methionine cycle, SAH is hydrolyzed into Hcy and adenosine through SAHH. Due to the
Therapeutic Advances in Chronic Disease 10

comparable $K_m$ of SAH and adenosine for SAHH, the reaction is highly reversible.27 This means that any increase in Hcy generation is associated with a similar increase in adenosine. In recent studies, the serum levels of Hcy and adenosine increased in patients with CAD, and showed a linear correlation.28 As an endogenous signaling molecule with a short half-life (0.6–1.5 s), serum adenosine levels are low in physiological conditions. However, ischemia, hypoxia, inflammation, stress, and other factors promote adenosine generation and its levels in the serum.29,30 A large number of in vitro and in vivo experiments also indicate that adenosine has a cardioprotective effect through its ability to induce coronary artery vasodilation, scavenge oxyradicals, prevent platelet activation, and improve cholesterol homeostasis.31,32 Adenosine acts as a metabolite of the methionine cycle and plays an opposing role to UA, Hcy and ADMA, to maintain physiological homeostasis.

Due to the close relationship between ADA and lymphocytes, ADA assays are commonly used to assist the diagnosis of diseases associated with cellular immunity or lymphocyte proliferation, particularly in tuberculosis and liver disease. As an important enzyme in the methionine cycle, ADA irreversibly catalyzes the deamination of adenosine to inosine, and inosine is subsequently metabolized into UA. ADA plays an important role in regulating the balance of adenosine, UA, Hcy and ADMA, to maintain physiological homeostasis.

The relationship between ADA and cardiovascular disease is of concern. Tang and coworkers summarized the impact of ADA on the cardiovascular system in the form of a medical hypotheses, including ADA-mediated inflammatory processes, the generation of superoxide radicals, the impact of ADA on myocardial ischemia and its potential clinical value.33 Unfortunately, serum

### Table 3. Associations between serum ADA activity and presence of CAD.

| Adjustment models                                                                 | OR 95%CI          | p         |
|----------------------------------------------------------------------------------|-------------------|-----------|
| Model 1: Crude, no adjustment                                                    | –                 | <0.001    |
| Model 2: Adjusting for age, sex, BMI, smoking, drinking, hypertension, and diabetes status | 0.873 (0.861–0.885) | <0.001    |
| Model 3: Adjusting for FBG, TG, TC, HDL-C, LDL-C, UA, SCr and ALT                | 0.862 (0.851–0.873) | <0.001    |
| Model 4: Adjusting for BMI, FBG, TG, TC, HDL-C, LDL-C, UA, ALT, smoking, drinking, hypertension, and diabetes status | 0.867 (0.855–0.880) | <0.001    |
| Model 5: Adjusting for BMI, FBG, TG, TC, HDL-C, LDL-C, UA, ALT, smoking, drinking, hypertension, diabetes statuses and medications | 0.852 (0.839–0.865) | <0.001    |

ADA, adenosine deaminase; ALT, alanine aminotransferase; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; SCr, serum creatinine; TC, total cholesterol; TG, triglyceride; UA, uric acid.

### Figure 2. Serum ADA activity in patients with different types of CAD.
Serum ADA activity in patients with MI (9.77 ± 3.80 U/l, $n = 1873$) was significantly lower in patients with SAP (10.26 ± 3.70 U/l, $n = 1332$) and UAP (10.25 ± 3.22 U/l, $n = 2007$). No differences between stable angina and unstable angina were observed. ADA levels are described as mean ± SD.

ADA, adenosine deaminase; CAD, coronary artery disease; MI, myocardial infarction; SAP, stable angina pectoris; SD, standard deviation; UAP, unstable angina pectoris.
ADA activity in patients with cardiovascular disease was not measured to verify this hypothesis.

Jyothy and coworkers measured serum ADA activity in 50 Indian patients with MI and 50 healthy controls using the colorimetric methods described by Giusti and Galanti in 1984. The results indicated that ADA activity (units of μ/l in the article) increased in patients with MI. We believe that ADA does not act directly on the target organs (endothelium) as is the case for metabolites in the methionine cycle. The effects of ADA on the endothelium are mediated by upstream and downstream metabolites and the activity of ADA influences the feedback of these metabolites. In addition, Khodadadi and colleagues demonstrated the production of an indophenol complex from the ammonia liberated from adenosine through spectrophotometry. They further determined ADA activity based on the Bertholet reaction. To date, the reference value of ADA activity in the healthy population is generally less than 19.6UI/l. In the study of Khodadadi et al., ADA activity in the control group was close to the upper limit of the reference values. We believe that bias exists, which may be caused by the small sample size of the control group (n = 55). To clearly explore the relationship between serum ADA activity and the presence of CAD, a large case-control sample size was required. A small sample size may have led to study bias. We included 5212 CAD patients and 4717 controls, and evaluated the association between serum ADA activity and the presence of CAD. Our findings did not agree with the results of the study of Jyothy et al., most likely due to their limited sample size. In this study, serum ADA activity was significantly attenuated in patients with CAD (10.08 ± 3.57 U/l) compared with controls (11.71 ± 4.20 U/l, p < 0.001). Patients with MI maintained the lowest levels of serum ADA activity (9.77 ± 3.80 U/l) compared with patients with stable and unstable angina. In addition, the elevated serum ADA activity in DM patients was consistent with previous studies, and our results demonstrate that the FBG levels positively correlate with serum ADA activity. As an enzyme related to substance metabolism, the activity, synthesis, and catabolism of ADA must be achieved through neuro–humoral regulation. When cardiovascular events occur, the body maintains higher levels of adenosine to protect
the cardiovascular system. Under these conditions, ADA activity undergoes negative-feedback regulation and is downregulated to reduce adenosine catabolism. The increasing levels of adenosine subsequently enhance cardiovascular protection. The mechanisms explaining the loss in serum ADA activity in patients with CAD may be complex and require further investigation.

This study should be considered as a preliminary report and does have some limitations. First, a control group comprised of age- and sex-matched individuals with no signs or symptoms of CAD and normal routine blood tests should be included. In addition, coronary angiography was not performed in all control patients. Second, the study was retrospective and could not dynamically observe changes in serum ADA activity in CAD patients. This was not conducive to studying the relationship between serum ADA activity and disease progression. Third, we tested ADA activity once per sample, and biological variations in enzyme activity may have affected the experimental accuracy. ADA activity is a relatively reliable clinical test index that can be traced to the international standard reference substance BCR647. The results of the automatic biochemical analyzers are reliable and repeatable. Instruments and reagents are calibrated and quality controls are performed prior to testing. In our clinical laboratory, the variable coefficient of repeatability of ADA activity was less than 5%. Finally, the reasons for the loss of ADA activity were not defined in CAD patients in this study. We categorized CAD into SAP, UAP and MI, according to the disease subtypes, and classified CAD according to the number of diseased vessels. As a more severe manifestation of CAD progression, serum ADA activity in patients with MI significantly decreased compared with patients with CAD. A similar association was not observed for other subtypes and the number of diseased vessels. ADA activity may therefore play an important role in the prevention of CAD, but further studies to clarify the mechanism(s) of its activity are now required.

In conclusion, our results suggest that the serum ADA activity is significantly lower in patients with CAD, particularly in patients with MI. ADA activity was affected by blood glucose, blood pressure, and living habits. This may reveal new roles of ADA in cardiovascular disease. ADA assays have been widely performed in clinical laboratories. Further clarifying the relationship between serum ADA activity and CAD is significant for disease prevention, control, and therapeutic monitoring. Prospective studies will also be performed in future studies.

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

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Conflict of interest statement
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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