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Serum Adenosine Deaminase as a Useful Marker to Estimate Coronary Artery Calcification in Type 2 Diabetes Mellitus Patients

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Abstract

We investigated the association between serum adenosine deaminase and coronary artery calcification (CAC) in type 2 diabetes mellitus (T2DM) patients. The cross-sectional study included 459 patients with T2DM, the clinical and laboratory tests were performed, and all T2DM patients were separated into the 3 groups based on the tertile of serum adenosine deaminase levels. In the baseline data, the CAC score had statistically significant differences between the 3 groups ($p < 0.001$). Serum adenosine deaminase levels were positively correlated with CAC score in T2DM patients ($r = 0.355$, $p < 0.001$). The results of multiple linear regression analysis showed that serum adenosine deaminase was independent positively correlated with CAC score in T2DM patients ($r = 0.255$, $p < 0.001$). Receiver-operating characteristic curve analysis showed that area under curve was 0.750 to identify T2DM patients with CAC. Serum adenosine deaminase levels are correlated with CAC scores in T2DM patients, clinically, serum adenosine deaminase should be considered as an underlying marker to determine the severity of atherosclerosis in T2DM patients.

Keywords

serum adenosine deaminase, type 2 diabetes mellitus, coronary artery calcification, cardiovascular disease

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Introduction

Cardiovascular disease is a major complication in patients with type 2 diabetes mellitus (T2DM), and T2DM patients increase the 2-3 folds risk of cardiovascular disease.[1] Contemporary data have showed that diabetes mellitus is close related with the risk of cardiovascular mortality.[2] Clinically, coronary artery calcification (CAC) has been acknowledged to the deposition of calcium in coronary arteries, CAC is highly correlated to the occurrence and progress of atherosclerosis,[3] and the CAC score is an outstanding predictor of cardiovascular events in patients with T2DM.[4]

Adenosine deaminase is a widely catalytic enzyme that catalyzes adenosine deamination to inosine.[5] Serum adenosine deaminase is a clinical biomarker in multiple diseases, such as hepatic injury, hemolytic anemia and tuberculous disease.[6] Recent studies have suggested that serum adenosine deaminase levels are associated with patients with recent-onset schizophrenia, cutaneous anthrax and chronic tonsillitis.[7-9] Recently, due to the fact increased serum adenosine deaminase levels in T2DM patients with adverse glycemic control,[10] then, the poor glycemic control is a risk factor for atherosclerosis in T2DM

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Table 1. Clinical Parameters Are Separated According to the Tertile of Serum Adenosine Deaminase.

	<9 n = 150	9-13 n = 174	>13 n = 135	p-values
Gender (Male, n %)	94 (62.7)	113 (64.9)	94 (69.6)	0.455
Age(yr)	46.8 ± 8.2	47.8 ± 9.7	48.4 ± 9.7	0.313
Body mass index (kg/m^2)	21.7 ± 2.2	23.0 ± 2.5	23.0 ± 2.4	<0.001
Smoking, n (%)	37 (24.7)	39 (22.4)	30 (22.2)	0.856
Alcohol use, n (%)	15 (10.0)	15 (8.6)	10 (7.4)	0.740
Hypertension, n (%)	40(26.7)	54 (31.0)	46 (34.1)	0.391
Hyperlipidemia, n (%)	75 (50%)	75 (43.1%)	61 (45.2%)	0.452
Duration of diabetes (years)	7.7 ± 7.5	9.3 ± 8.6	10.1 ± 9.4	0.048
Insulin, n (%)	26 (17.3)	44 (25.3)	40 (29.6)	0.046
Aspirin, n (%)	110 (73.3%)	109 (62.6%)	84 (62.6)	0.070
ACEI/ARBs, n (%)	135 (90.0)	166 (95.4)	124 (91.9)	0.167
High-sensitivity C-reactive protein (mg/L)	3.0 ± 2.1	3.2 ± 2.2	4.2 ± 2.2	<0.001
Fasting blood glucose (mmol/L)	6.8 ± 3.1	7.9 ± 2.6	9.9 ± 3.7	<0.001
Glycosylated hemoglobin (%)	7.8 ± 4.6	8.2 ± 4.3	10.5 ± 4.1	<0.001
Coronary artery calcification scores	25.6 ± 56.1	32.0 ± 82.1	143.6 ± 136.4	<0.001

ACEI = angiotensin-converting enzyme inhibitor, ARBs = angiotensin-2 receptor blockers.

patients.[11] Thus, we investigated the association between serum adenosine deaminase and CAC in T2DM patients.

Methods and Materials

T2DM Patients

459 patients with type 2 diabetes who referred to WHO criteria[12] were diagnosed, and all T2DM patients were separated into the 3 groups based on the tertile of serum adenosine deaminase levels, in these T2DM patients, patients were subjected to computed tomography scan. This study was approved by Ethics Committee of Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, and our study obtained the informed consent of the patients. Exclusion criteria: patients with acute or chronic infections, tuberculous disease, cancer, liver damage, rheumatic disease, renal insufficiency, pregnancy and hematologic disease were excluded.

Blood Analytes

Blood samples were drawn in the morning after fasted overnight in all patients. In total, serum was separated to test laboratory indexes by high-speed centrifugation with 3,000 rpm at room temperature for 10 minutes, and further biochemical tests were performed.

Clinical Materials

Physical examinations were carried out. Blood pressure was assessed by using a mercury sphygmomanometer. The clinical materials such as body mass index, medication use and diabetes duration et al were obtained in electronic medical records.

The Measurements for CAC Score

Image tests were implemented by computed tomography scan with a gantry rotation speed of 0.4 s/rotation, 120 kV and 100 mA, the CAC score was estimated by computed tomography scan, and CAC score was categorized on the basis of following grades: $\leq$10 (minimal or none); 11-100 (mild calcification); 101-400 (moderate calcification); 401-1000 (severe calcification); and greater than 1,000 (extensive calcification).[13]

Statistical Analysis

We used SPSS version 20.0 statistical software to assess the data. The data are shown as mean and standard deviation or percentage. Chi-square test and One-ANOVA were used to determine the differences between the 3 groups. The correlations were made by Pearson correlation test. We used multiple linear regression analysis to analyze the further correlation between serum adenosine deaminase and CAC score. Receiver-operating characteristic curve analysis was selected to assess the performance to identify T2DM patients with CAC form all patients. The p values <0.05 were considered statistically significant.

Results

Table 1 provided all T2DM patients' physical demographic, clinical materials and metabolic parameters, these data were separated into 3 groups on the basis of the tertile of serum adenosine deaminase levels, the CAC score had statistically significant differences between the 3 groups ($p<0.001$), and the statistically significant differences were observed in body mass index, duration of diabetes, insulin use, high-sensitivity C-reactive protein, fasting blood glucose and glycosylated hemoglobin between the 3 groups.

Table 2. The Serum Adenosine Deaminase Is Independently Correlated With CAC Scores in T2DM Patients in Multiple Linear Regression Analysis.

	Unstandardized cofficients		Standardized cofficients		
	B	Std Error	Beta	t	P-value
Body mass index	0.282	0.070	0.165	4.035	<0.001
Aspirin	-0.810	0.364	-0.091	-2.228	0.026
Fasting blood glucose	0.181	0.060	0.144	3.037	0.003
Glycosylated hemoglobin	0.087	0.043	0.092	2.029	0.043
High-sensitivity C-reactive protein	0.403	0.078	0.213	5.189	<0.001
Coronary artery calcification scores	0.010	0.002	0.255	5.695	<0.001

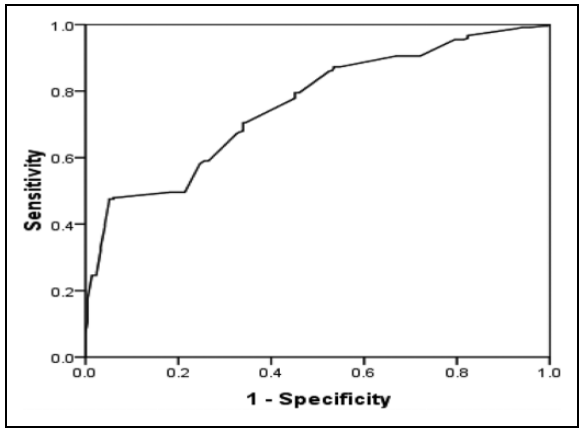

Figure 1. Receiver-operating characteristic curve analysis of serum adenosine deaminas in identifying T2DM patients with CAC.

In the correlation analyses, serum adenosine deaminase levels were positively correlated with body mass index, fasting blood glucose, glycosylated hemoglobin in patients with T2DM ($r = 0.134$, $p = 0.004$; $r = 0.382$, $p < 0.001$; and $r = 0.237$, $p < 0.001$), moreover, there was an additional positively correlation between serum adenosine deaminase and high-sensitivity C-reactive protein ($r = 0.227$, $p<0.001$), and serum adenosine deaminase was observed to be positively correlated with CAC score in T2DM patients ($r = 0.355$, $p < 0.001$).

Table 2 provided the results of multiple linear regression analysis, we adjusted the variables including gender, age, body mass index, smoking, alcohol use, hypertension, duration of diabetes, medication use, high-sensitivity C-reactive protein, fasting blood glucose, glycosylated hemoglobin, and hyperlipidemia in the multiple linear regression analysis, afterward, the serum adenosine deaminase was independently positively correlated with CAC score in patients with T2DM ($r = 0.255$, $p < 0.001$).

Patients who the CAC score (>10) were defined as CAC positive, so receiver-operating characteristic curve analysis was used to identify the T2DM patients with CAC from all patients, the results showed that area under curve was 0.750 with sensitivity of 0.699 and specificity of 0.657, (Figure 1) and the cutoff values of serum adenosine deaminase were 10.2 in identifying T2DM patients with CAC.

Discussion

Subclinical atherosclerosis is a clinical disadvantage factor for cardiovascular disease in patients with T2DM, and the CAC is an independently predictor for the overall load of atherosclerosis in persons with and without obstructive coronary artery disease.[14] In this study, we found the link between serum adenosine deaminase and fasting blood glucose and glycosylated hemoglobin in T2DM patients, the results are consistent with previous study,[15] Interesting, we observed an additional correlation between serum adenosine deaminase and CAC score in T2DM patients.

Low-grade inflammation is active in T2DM patients,[16] inflammatory components have been observed in the pathogenesis of atherosclerosis in T2DM, and population-based study has found that inflammation markers are correlated with intermediate cardiovascular endpoints, especially in intima-media thickness,[17] and the inflammatory parameters are associated with coronary calcification.[18,19] Furthermore, experimental study has suggested that inflammation mediated-molecular and cellular pathways can promote atherosclerosis, key inflammatory mechanism is important in atherogenesis as an active process.[20] More direct evidence has been suggested that the increased adenosine deaminase activity is observed as a marker in early stage of atherosclerosis, which contributes to its progression and development of CAC.[21] Thus, inflammation may be a major mechanism to elevate serum adenosine deaminase in T2DM patients with CAC. As expected, the association between adenosine deaminase and inflammation have been reported, inhibition of ADA activity can contribute to relieve inflammation and cell proliferation.[22] There are several reports indicating that the increase of serum adenosine deaminase due to the stimulation of inflammation. An association of serum adenosine deaminase with systemic inflammation is observed in experimental colitis,[23] and serum adenosine deaminase activity is elevated in inflammatory diseases such as acute lymphoblastic leukemia, autoimmune hepatitis and rheumatic disease.[24-27] Serum adenosine deaminase has been reported to be an inflammatory marker in rheumatoid arthritis.[28] In another study showed that serum adenosine deaminase, an endogenous anti-inflammatory metabolite, is elevated in response to inflammatory status resulted by adipose tissue in obesity.[29] Serum adenosine deaminase levels have been found to be

increased in patients with Inflammatory bowel disease, and serum adenosine deaminase is a useful biomarker for assessment of intestinal inflammation.[30] It is well established in the literature that a significantly correlation between serum adenosine deaminase and C-reactive protein has been suggested in patients with chagas disease,[31] supporting the view, our data also showed the link between serum adenosine deaminase and high-sensitivity C-reactive protein in T2DM patients.

In another aspect, poor glycemic control increases the risk of coronary atherosclerosis plaque in T2DM patients, in return, adenosine deaminase can increase insulin sensitivity for glucose transport, and elevate the accessibility about 25% of GLUT4 to cell envelope for glucose transportation.[32] Therefore, long-term hyperglycemia also may increase the adaptation of adenosine in patients with T2DM, leading to an increased serum adenosine deaminase levels in T2DM patients with CAC.

We have to consider some limitations. First, our study is only a hospital-based cross-sectional design in general diabetic population, further study should be considered in community population. Second, the effects of anti-inflammatory treatment on serum adenosine deaminase levels were not evaluated in patients with T2DM. Third, use of hypoglycemic and lipid-lowering drugs may affect adenosine deaminase levels in patients with T2DM. Fourth, CAC is an estimated value, but a direct measure of coronary atherosclerosis,[33] thus, some patients with coronary atherosclerotic plaques may not be detected. In addition, our study did not examine the relationship between serum adenosine deaminase and clinical outcomes in T2DM patients with CAC.

Conclusions

In the light of our results, serum adenosine deaminase levels are positively correlated with CAC score in T2DM patients, serum adenosine deaminase may be a useful marker to assess the severity of atherosclerosis in patients with T2DM.

Author Contribution

Ming Yu and Hanyun Zhou contributed to this study as co-first authors. Conceived and designed the study: Hongxia Shuai, Juan Ding and Ming Yu. Performed the experiments: Ming Yu, Hanyun Zhou and Qingan Li. Analyzed the data: Ming Yu, Hanyun Zhou. Contributed materials and analysis: Ming Yu, Ji Zhang. Wrote the manuscript: Ming Yu.

Declaration of Conflicting Interests

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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