

RESEARCH

The association between serum adenosine deaminase levels and Graves' disease

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Abstract

Background: Adenosine deaminase (ADA) is essential for the differentiation and maturation of lymphocytes, while lymphocytes infiltration in thyroid tissue is a vital pathological feature of Graves' disease (GD). The aim of the present study was to compare the concentration of ADA between healthy controls (HC) and patients with GD, and evaluate the association between ADA and GD.

Methods: A total of 112 GD patients and 77 matched HC were enrolled in this study. Each participant was examined for thyroid hormones and autoantibodies, ADA concentration, and thyroid ultrasonography.

Results: Serum ADA levels in GD patients were significantly higher than that in HC subgroup ($P < 0.001$). In GD patients, serum ADA levels were positively associated with serum-free triiodothyronine (FT3), free thyroxine (FT4), thyroid peroxidase antibody (TPOAb), thyroid-stimulating hormone receptor antibody (TRAb) levels, and total thyroid gland volume (thyroid VolT) and negatively associated with serum thyroid-stimulating hormone receptor (TSH) levels (all $P < 0.05$). There were no similar correlations in the HC subgroup. Multiple linear regression analysis suggested that serum TSH, FT3, and ADA levels played an important role in serum TRAb levels.

Conclusions: Our results demonstrated that serum ADA levels were closely associated with GD.

Key Words

- ▶ adenosine deaminase
- ▶ Graves' disease
- ▶ thyroid-stimulating hormone receptor antibody

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Introduction

Graves' disease (GD) characterized by diffuse goiter, thyrotoxicosis, and ophthalmopathy is an organ-specific autoimmune disease, with a prevalence of about 1% (1). GD is a multifactorial disease that genetic predisposition, environmental factors, and immune system dysfunction are all implicated in GD pathogenesis, among which immune system dysfunction plays the most important role (2). As an autoimmune disease, relapsing cases of GD are common, and remission time is uncertain, which may create difficulties in treating this disease (3). Treatments for GD include oral antithyroid drugs, radioactive iodine therapy (RAI), and surgery, but each treatment regimen has the possibility of side effects (4). From this statement,

further understanding of the mechanism of immune system dysfunction in GD pathogenesis and exploring new therapeutic targets are of great importance for improving the prognosis of GD patients.

Adenosine deaminase (ADA) is a polymorphic enzyme which is expressed in all human tissues. In addition to regulating adenosine concentration by catalyzing the irreversible deamination of adenosine to inosine (5), ADA is also essential for the differentiation and maturation of T lymphocytes and is a general marker of cellular immunity (6). In some autoimmune diseases, such as psoriasis (7), autoimmune hepatitis (8), inflammatory bowel disease (9), and rheumatic disease (10),

high serum concentrations of ADA were observed. Meanwhile, increased ADA activity was also observed in the peripheral blood leucocytes of patients with GD (11) and monocytes of patients with Hashimoto disease (12). Therefore, serum ADA level may have the potential to be an indicator for the evaluation and monitoring of GD. Moreover, in patients with psoriasis, treatment with propylthiouracil (PTU), a kind of oral antithyroid drugs, significantly reduced the epidermal ADA activity while improving psoriatic plaques (13). Based on this, we speculated that PTU might play a role in the treatment of hyperthyroidism partly by inhibiting the activity of ADA, so ADA might be a potential therapeutic target for GD. However, to our knowledge, there are no clinical studies that have explored the relationship between serum ADA levels and GD.

Therefore, the present study was designed to compare the concentration of ADA between HC and GD patients, and to evaluate the association between ADA and GD.

Methods

Participants' selection

This was a cross-sectional study, and a total of 112 GD patients at the inpatient department of the Second Affiliated Hospital of Nantong University between January 2021 and April 2021 were enrolled. GD was defined as: clinical symptoms of hyperthyroidism, characteristic of thyroid ultrasound images, and biochemical indicators (each criterion must be met: low thyroid-stimulating hormone – TSH, high free triiodothyronine – FT3, high free thyroxine – FT4, elevated TSH receptor antibody – TRAb) (14). The exclusion criteria were as follows: (1) complicated with other autoimmune diseases; (2) use of drugs in the past 3 months that affect immunity, i.e. steroids; (3) pregnancy, previous and current malignant tumors; and (4) abnormal liver function due to various causes. During the same period, the present study also included 77 age- and sex-matched healthy controls from the Department of Physical Examination Center. The study protocol was approved by the medical research ethics committee of the Second Affiliated Hospital of Nantong University, and completely complied with the Declaration of Helsinki. Upon enrollment, each subject provided a written informed consent.

Basic data collection

Clinical data including age, sex, smoking, medical history, and anthropometry parameters were obtained from all

participants through interviews and examinations by experienced physicians. BMI was calculated as the weight (kg)/height (m²). After resting for at least 30 min, blood pressure of each participant was measured by a standard mercury sphygmomanometer, and the average of three recordings were recorded.

Laboratory examination

After an overnight fasting, venous blood samples were drawn to measure laboratory parameters. Serum ADA concentrations were measured with an automated biochemical analyzer (Model 7600, Hitachi). Serum TSH, FT3, FT4, TRAb, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb) levels were determined using chemiluminescence methods with an immunoassay system (Dxi800, Beckman Coulter).

Statistical analysis

Clinical variables were shown for all subjects, GD subgroup, HC subgroup, and for the three subgroups of GD patients according to ADA tertiles. The mean \pm s.d, median (25 and 75% interquartile), and frequency (percentage) were adopted to describe continuous variables with normal and skewed distributions and categorical variables, respectively. We adopted Student's *t*-test to compare differences in normally distributed data, the Mann–Whitney test to compare differences in skewed distributed data, and the chi-square test to compare categorical data between GD subgroup and HC subgroup. One-way ANOVA, the Kruskal–Wallis test, and the chi-square test were used to compare differences in normally distributed data, skewed data, and categorical data among the three subgroups of GD patients based on ADA tertiles. Spearman's bivariate correlation analysis was constructed to evaluate the correlations of ADA levels with clinical parameters in GD subgroup and HC subgroup, respectively. Since the distribution of serum TRAb level was skewed, a natural logarithm transformation (ln) was applied to achieve a normal distribution. Subsequently, multiple linear regression analysis was undertaken to identify independent contributors to ln (TRAb). Furthermore, receiver operating characteristic (ROC) analysis was conducted to analyze the ability of ADA levels to indicate GD cases, and the corresponding cut-off value was provided. Data analyses were performed using SPSS statistical software 18.0 (IBM SPSS Inc.). A value of *P* < 0.05 was considered to be statistically significant.

Results

Basic characteristics

Table 1 displays the clinical characteristics of the participants. Compared with HC, GD patients had higher ADA concentrations, FT3 levels, FT4 levels, TPOAb levels, TgAb levels, TRAb levels, total thyroid gland volume (thyroid VoIT), lower BMI, and TSH levels (all $P < 0.001$). There were no differences in age, proportion of males, proportion of smoking, and systolic/diastolic blood pressure ($P > 0.05$) between the two subgroups.

GD patients were then divided into three subgroups according to the ADA tertiles, and clinical indicators were compared between the subgroups. As shown in Table 2, along with the ADA tertile ascending, age, FT3 levels, FT4 levels, TPOAb levels, TRAb levels, and thyroid VoIT significantly increased (P for trend < 0.05), whereas diastolic blood pressure (DBP) and TSH levels decreased (P for trend < 0.05). However, proportion of males, proportion of smoking, proportion of relapsed case, proportion of antithyroid treatment, BMI, systolic blood pressure (SBP), and TgAb levels did not show any difference among the tertiles of ADA levels (P for trend > 0.05).

Relationship between ADA and clinical parameters

Table 3 shows that in GD subgroup, serum ADA levels were positively associated with age, FT3 levels, FT4 levels, TPOAb levels, TRAb levels, and thyroid VoIT ($r = 0.298, r = 0.378, r = 0.379, r = 0.209, r = 0.518, r = 0.421$,

respectively, all $P < 0.05$) and negatively associated with DBP and TSH levels ($r = -0.248, r = -0.262$, respectively, both $P < 0.05$). However, there were no significant correlations between ADA levels and male, smoking, relapsed case, antithyroid treatment, BMI, SBP, or TgAb levels (all $P > 0.05$). In HC subgroup, only a significant positive correlation between serum ADA levels and age ($r = 0.273, P < 0.05$) was observed.

Multiple linear regression analysis with ln (TRAb) as the dependent variable in GD subgroup

To explore which parameters were independent contributors to TRAb, we constructed a multiple linear regression analysis. After adjusting for age, male, smoking, relapsed case, antithyroid therapy, BMI, SBP, DBP, FT4, TPOAb, TgAb and thyroid VoIT, TSH levels ($\beta = -0.470, t = -2.951, P = 0.004$), FT3 levels ($\beta = 0.064, t = 2.501, P = 0.005$), and ADA levels ($\beta = 0.118, t = 4.478, P < 0.001$) were independently correlated with ln (TRAb) (Table 4).

ROC analysis to explore the cut-off ADA value to diagnose GD cases

ROC analysis was further conducted to explore the cutoff ADA value to indicate confirmed GD cases. The optimal cutoff value of ADA to indicate GD was 10 U/L. The corresponding area under the curve (AUC) to indicate GD was 0.808 (95% CI: 0.746–0.869), its Youden index

Table 1 Clinical characteristics of the study participants.

Variables	Total	GD	HC	P value
<i>n</i>	189	112	77	
Age (years)	47.7 ± 13.79	47.65 ± 14.37	47.78 ± 12.99	0.950
Male, <i>n</i> (%)	33 (17.5)	17 (15.2)	16 (20.8)	0.211
Smoking, <i>n</i> (%)	7 (3.7)	4 (3.6)	3 (3.9)	0.599
BMI (kg/m ²)	22.77 ± 3.37	22.04 ± 3.24	23.83 ± 3.29	<0.001
SBP (mmHg)	123.0 (115.0–138.5)	123.5 (116.3–141.5)	122.0 (113.5–135.0)	0.240
DBP (mmHg)	77.10 ± 10.58	76.20 ± 10.49	78.40 ± 10.65	0.160
TSH (mIU/L)	0.01 (0.01–1.71)	0.01 (0.01–0.01)	1.91 (1.34–2.79)	<0.001
FT3 (IU/L)	5.85 (4.91–14.74)	13.04 (7.40–20.03)	4.88 (4.48–5.31)	<0.001
FT4 (IU/L)	14.81 (10.83–46.26)	40.56 (20.72–53.65)	10.95 (9.88–11.62)	<0.001
TPOAb (IU/mL)	40.50 (1.60–436.98)	218.15 (9.70–671.58)	1.3 (0.6–9.2)	<0.001
TgAb (IU/mL)	1.7 (0.1–20.9)	3.9 (0.5–36.2)	0.1 (0.1–3.1)	<0.001
TRAb (IU/L)	4.13 (0.80–12.16)	8.54 (4.12–20.46)	0.80 (0.58–0.80)	<0.001
Thyroid VoIT (mL)	24.23 (16.78–39.50)	30.39 (20.52–45.13)	16.63 (20.52–20.21)	<0.001
ADA (U/L)	9 (7–12)	11 (9–14)	8 (7–9)	<0.001

Normally distributed values in the table are given as the mean ± s.d., skewed distributed values are given as the median (25 and 75% interquartiles), and categorical variables are given as frequency (percentage). The two independent sample *t*-test, the Mann-Whitney test and the chi-square test were conducted to determine *P* values for normally distributed continuous variables, skewed continuous variables, and categorical variables, respectively. ADA, adenosine deaminase; FT4, free thyroxine; FT3, free triiodothyronine; SBP/DBP, systolic/diastolic blood pressure; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TRAb, TSH receptor antibody; TSH, thyroid-stimulating hormone; thyroid VoIT, total thyroid gland volume.

Table 2 Clinical characteristics of GD patients based on ADA tertiles.

Variables	T1	T2	T3	P for trend
ADA (U/L)	<10	10–12	>12	
<i>n</i>	37	35	40	
Age (years)	44.05 ± 15.19	45.46 ± 12.21	52.90 ± 14.14	0.013
Male, <i>n</i> (%)	6 (16.2)	7 (20.0)	4 (10.0)	0.473
Smoking, <i>n</i> (%)	2 (5.4)	1 (2.9)	1 (2.5)	0.761
Relapsed case, <i>n</i> (%)	6 (16.7)	7 (20.6)	7 (17.5)	0.905
Antithyroid treatment				
None, <i>n</i> (%)	25 (69.4)	19 (55.9)	28 (70.0)	0.622
Propylthiouracil, <i>n</i> (%)	2 (5.6)	2 (5.9)	3 (7.5)	0.622
Methimazole, <i>n</i> (%)	9 (25.0)	13 (38.2)	9 (22.5)	0.622
BMI (kg/m ²)	22.10 ± 3.47	21.99 ± 3.09	22.03 ± 3.26	0.989
SBP (mmHg)	125.0 (116.0–141.0)	121.0 (114.0–134.0)	126.5 (117.0–146.0)	0.448
DBP (mmHg)	79.73 ± 10.96	76.60 ± 10.37	72.58 ± 9.13	0.010
TSH (mIU/L)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.009
FT3 (IU/L)	8.88 (5.70–14.45)	14.10 (8.53–19.92)	17.10 (9.51–24.67)	0.002
FT4 (IU/L)	22.73 (13.59–335.58)	43.18 (29.95–49.83)	47.61 (28.79–59.94)	0.002
TPOAb (IU/mL)	114.2 (15.1–335.6)	258.9 (5.70–645.0)	350.40 (58.8–905.2)	<0.001
TgAb (IU/mL)	1.8 (0.3–22.0)	4.9 (0.5–23.9)	7.9 (0.5–52.5)	0.358
TRAb (IU/L)	4.37 (1.70–6.96)	7.93 (4.84–15.00)	19.93 (9.08–26.20)	<0.001
Thyroid VolT (mL)	24.16 (13.96–34.67)	29.0 (19.89–43.19)	39.25 (25.20–52.46)	0.005

ANOVA, the Mann–Whitney test, and the chi-square test were conducted to determine *P* values for normally distributed continuous variables, skewed continuous variables, and categorical variables, respectively.

was 0.475, its sensitivity was 67.0%, and its specificity was 80.5% (Fig. 1).

Discussion

In the present study, we compared serum ADA levels and explored the association between serum ADA levels and GD among a cohort medium-sized of Chinese participants. The main findings of this study are as follows: first, compared

to HC, patients with GD had relatively higher serum ADA levels; second, along with the ADA tertile ascending, serum FT3 levels, FT4 levels, TPOAb levels, TRAb levels, and thyroid VolT gradually increased in GD patients; third, in GD patients, serum ADA levels were positively associated with serum FT3 levels, FT4 levels, TPOAb levels, TRAb levels, and thyroid VolT, and negatively associated with serum TSH levels; while there were no similar correlations observed in HC; fourth, ADA was independently positively associated with ln (TRAb) in GD patients; fifth, the optimal cut-off value of ADA to indicate GD was 10 U/L, and its corresponding sensitivity and specificity were 67.0 and 80.5%, respectively.

Circulation TRAb is the hallmark of GD. By binding to the TSH receptor, TRAb enhances the production of cAMP in thyrocytes, thereby promoting the release of TSH and the growth of thyrocytes, and eventually causing hyperthyroidism and goiter (15). Therefore, the production of TRAb plays the key role in the onset and progression of GD. Dendritic cells (DCs) present antigens to CD4⁺ T cells which then stimulate the

Table 3 Relationship between ADA and clinical parameters.

Variables	GD		HC	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age	0.298	0.001	0.273	0.016
Male	0.043	0.653	0.118	0.305
Smoking	-0.065	0.496	-0.150	0.192
Relapsed case	-	-	0.012	0.905
Antithyroid treatment	-	-	-0.043	0.656
BMI	0.000	0.999	0.044	0.706
SBP	0.117	0.218	0.117	0.313
DBP	-0.248	0.009	0.052	0.652
TSH	-0.262	0.005	-0.031	0.789
FT3	0.378	<0.001	-0.008	0.942
FT4	0.379	<0.001	0.121	0.296
TPOAb	0.209	0.029	-0.087	0.510
TgAb	0.095	0.322	-0.055	0.689
TRAb	0.518	<0.001	-0.079	0.567
Thyroid VolT	0.421	<0.001	0.386	0.052

r, Spearman's correlation coefficient.

Table 4 Multiple linear regression analysis with ln (TRAb) as the dependent variable in GD subgroup.

Variables	B (95% CI)	<i>t</i>	<i>P</i>
TSH	-0.470 (-0.786, -0.154)	-2.951	0.004
FT3	0.064 (0.020, 0.109)	2.501	0.005
ADA	0.118 (0.066, 0.171)	4.478	<0.001

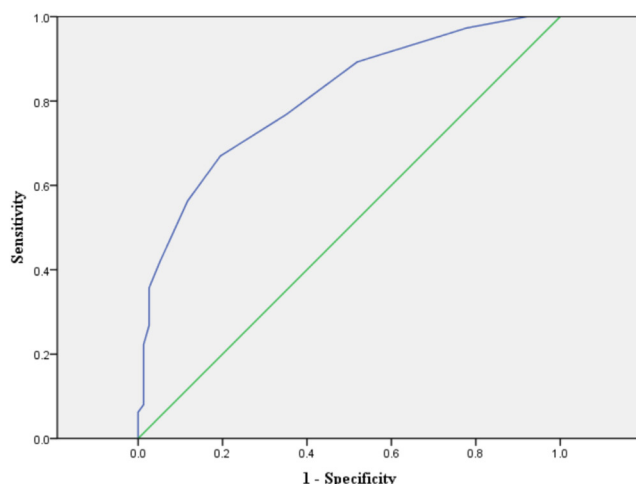


Figure 1
ROC analysis to analyze the ability of ADA to indicate GD.

persistent activation and expansion of B cells to produce TRAb (16). When subjected to invading pathogen and foreign antigen, immature DCs (iDCs) which are widely distributed among peripheral tissues can be activated. Casanova *et al.* cultured human iDCs *in vitro*, and added ADA to the culture medium to enhance the maturation of DCs and render DCs that are more immunogenic (17). For CD4⁺ T cells, ADA can interact with CD26 on the surface of T cells to trigger co-stimulatory signals of human T cells, suggesting that ADA is an important modulator of CD4⁺ T cell differentiation (18). Hence, ADA may participate in the onset and progress of GD by stimulating DCs and T cells to promote the production of TRAb. Consistent with this speculation, the present study found that ADA was an independent contributor to In (TRAb) in GD patients. In addition, ADA can significantly increase the production of T helper-1 cells and proinflammatory cells, such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), which may contribute to the exacerbation of GD (19).

In this study, we found that serum ADA levels in GD patients were significantly higher than those in HC, and that serum ADA levels in GD patients were closely related to thyroid function. Untreated hyperthyroidism in GD patients can cause a sort of complications, including weight loss, osteoporosis, thrombosis, atrial fibrillation, brittle fracture, cardiac hypertrophy, and congestive heart failure and so on (20, 21, 22). In the hypermetabolic state of hyperthyroidism, the increase of intracellular ATP and oxygen consumption and the dysfunction of mitochondrial respiratory chain generate the imbalance of redox balance,

resulting in the over-production of reactive oxygen species (ROS) in peripheral tissues (23). A prospective study that enrolled 21 GD patients revealed that oxidative stress indexes were associated with thyroid function, and restoration of euthyroidism could significantly decreased oxidative stress indexes (24). Thus, excessive oxidative stress may be the pathophysiology basis for the occurrence of complications related to GD. Baldissarelli *et al.* established a hyperthyroid rat model by levothyroxine administration and found that ADA activity in the platelets of the hyperthyroid rat was significantly higher than controls, and ADA levels were positively associated with ROS levels (25). Multiple studies have confirmed the correlation between serum ADA levels and ROS in a variety of diseases (26), and some studies have also confirmed that inhibition of ADA can significantly reduce the production of ROS *in vivo* (27). The mechanism behind this connection is that ADA can accelerate the differentiation and maturation of T lymphocytes (6). Activated T lymphocytes can produce inflammatory cytokines, thus stimulating macrophage and neutrophil activations, eventually leading to excess production of ROS (28). Thus, ADA may be the bridge linking hyperthyroidism, oxidative stress, and GD complications.

Growing evidences have shown that inhibition of ADA is beneficial for ameliorating some diseases. Pentostatin is a potent ADA inhibitor that, when applied to atherosclerotic mice, exerted anti-atherosclerotic effects by reducing macrophage accumulation and improving endothelial function (29). In hyperthyroidism pig, the administration of pentostatin significantly increased adenosine levels, enhanced A1 adenosinergic system, which in turn exerted negative inotropic effects and might ultimately improve myocardial ischemia (30). Moreover, a review published in 2020 pointed out that multiple commonly used drugs such as simvastatin, metoprolol, and aspirin could significantly inhibit ADA activity at conventional doses (31). Therefore, inhibition of ADA in patients with GD is promising and simple to implement.

However, several limitations of our study were unavoidable. First, as a cross-sectional study, a cause-effect relationship between serum ADA levels and GD cannot be concluded. Second, the generalizability of our study is limited due to that all the subjects enrolled in this study were Chinese. Third, the immune function and oxidative stress indexes of GD patients were not measured simultaneously in this study. Therefore, further research should be conducted to validate the results of our study and to address the above limitations.

Conclusions

In summary, this is the first study to demonstrate that serum ADA levels are closely associated with GD. Elevated ADA may be involved in the pathogenesis of GD and the development of complications in GD patients. Future studies need to focus on evaluating the potential of ADA as a target for GD surveillance and treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Availability of data and materials

The current data are available to all interested researchers upon reasonable request.

Ethics approval and consent to participate

The study was approved by the institutional review board of Affiliated Hospital 2 of Nantong University and First People's Hospital of Nantong City, and written informed consent was obtained from all participants.

Author contribution statement

C L, W L, and F X participated in the design of the study, data collection, analysis of the data, and drafting of the manuscript. J S and C L conceived of the study, participated in its design and revised the manuscript. C L, X G, and X W participated in the analysis of the data and revised the manuscript. C L and Y W participated in data collection. All authors read and approved the final manuscript.

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