Original Article

Comparison of alpha 1- antitrypsin activity and phenotype in type 1 diabetic patients to healthy individuals

Atena Sadat Ghoreishi[1], Mehdi Mahmoodi[2], Alireza Khoshdel[3]

[1]Department of Clinical Biochemistry, Faculty of Para-Medicine, Jiroft University of Medical Sciences, Jiroft, [2]Department of Clinical Biochemistry, Afzalipoor Faculty of Medicine, Kerman University of Medical Sciences, Kerman, [3]Department of Clinical Biochemistry, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Abstract

Background and Aims: Alpha 1 antitrypsin (AAT) is an inhibitor of serine protease, which has shown anti-inflammatory reactions in a variety of diseases. It has been thought that that AAT plays a role in prolonging islet allograft survival, preventing the development of type 1 diabetes mellitus (T1DM), and hindering β-cell apoptosis of pancreas. In the current examination, the AAT activity in T1DM and healthy individuals was measured using enzymatic assay. **Methods:** The present study was conducted on 42 patients with T1DM who referred to the Diabetes Clinic of Rafsanjan, Kerman, Iran, and 42 healthy control individuals who were matched for age, sex and smoking habits. The serum trypsin inhibitory capacity (TIC) was assessed. Plasma samples were analyzed for phenotype, AAT concentration, blood glucose and lipid levels were measured. **Results:** The activity of plasma AAT and the serum TIC level of patients with T1DM (2.35 ± 0.34 μmol/min/ml) was significantly lower than healthy participants (3.36 ± 0.36 μmol/min/ml). The frequency of phenotype MM in healthy individual was 100%; and in T1DM patients, the prevalence of phenotype MM, MS and MZ was 61.9%, 23.8% and 14.3%, respectively ($P < 0.001$). **Conclusions:** It was concluded that that the lack of AAT may be related to the increased risk of T1DM developing.

Keywords: Alpha 1- antitrypsin, diabetes mellitus, phenotype, trypsin

Introduction

Type 1 diabetes mellitus (T1DM) is a disorder caused by chronic autoimmune destruction of pancreatic islet beta cells (β-cells). Improved function of residual β-cell reduces the rate of long-term complications and causes better glycemic control in patients.[1] Accordingly, a number of interventions have recently been conducted aimed to slow this inflammatory process within β-cell.[2,3] However, none of them have led to successful maintenance of residual β-cell function with minimal adverse effects. As an acute phase protein, AAT released mainly by pancreatic islets and hepatocytes under inflammatory situations and functions as the leading serine protease inhibitor in blood.[4] Furthermore, AAT participates in a variety of procedures, including reactive oxygen species toxicity, endothelial function and apoptosis, cell-mediated immunity/tolerance, neutrophil and endotoxin mediated inflammation, and reproduction.[5] Although AAT levels in patients with T1DM seems to be normal, its anti-protease function may reduce due to substantial non-enzymatic glycation and thereby, it might be inert.[6,7]

AAT level is genetically managed by alleles presented in various phenotypes, some of which are associated with protein deficit.

Address for correspondence: Dr. Alireza Khoshdel, Department of Clinical Biochemistry, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran.
E-mail: Alireza.khoshdel@gmail.com

Received: 16-03-2021 **Revised:** 24-07-2021
Accepted: 28-10-2021 **Published:** 18-03-2022

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_905_21

How to cite this article: Ghoreishi AS, Mahmoodi M, Khoshdel A. Comparison of alpha 1- antitrypsin activity and phenotype in type 1 diabetic patients to healthy individuals. J Family Med Prim Care 2022;11:1377-81.

Some studies have reported the anti-inflammatory impacts of AAT on expressing cathepsin G, proteinase 3 and neutrophil elastase (NE) by immune cells, and some others pointed to the suppressing effects of AAT on the producing some cytokines and chemokines, complement activation and infiltration of immune cells.[8] The recent studies revealed some mechanisms of activity of AAT which are unrelated to serpin activity.[4]

Recent evidence have showed that over-expression of AAT by gene delivery reduced insulitis and inhibited the progression of overt hyperglycemia in non-obese diabetic (NOD) mice.[9,10] Furthermore, studies have indicated prolongs pancreatic islet allograft survival by administration of pharmaceutical dosages of human AAT and shows islet-related cytoprotective impacts.[11] Although the underlying mechanism of AAT in producing these positive therapeutic outcomes is not fully understood, recent trials have shown that AAT treatment can inhibit caspase-3 activity and, as a result, protects pancreatic β-cell against apoptosis.[12] Given the ability of AAT in preventing both diabetes formation *(in vivo)* and β-cell apoptosis (in vitro), a logical assumption could be that AAT deficiency may be associated with a higher risk of developing diabetes. In line with this assumption, it has been documented that the plasma levels of AAT is essentially lower in patients with Type 1 diabetes (T1DM)[13] However, reported data about the relationship between the plasma levels of AAT and T1DM is insufficient. Consequently, we designed this examination to compare the AAT activity profile in T1DM patients to healthy individuals.

Materials and Methods

Materials

Acetic acid, Tris, CaCl2, HCl and DMSO were prepared from Merck Company, while A-N-benzyl-DL-arginine-p-nitroaniline (BAPNA), trypsin and Bovine serum albumin (BSA) were purchased from Sigma.

Patients

This cross-sectional study was conducted on type 1 diabetes patients who referred to the Diabetes Clinic of Rafsanjan, Kerman, Iran. The ethical committee of Rafsanjan university of Medical Sciences has approved this study under Ref. Number of IR.RUMS.REC.1394.13. Blood samples were obtained from T1DM (n = 42; 21 male, 21 female, age range 18–28 years) and healthy individual (n = 42; 21 male, 21 female, age range 18– 28 years). It was also a maximum of one year since the diagnosis of type 1 diabetes. The serum was separated during two hours of samples collection and stored at -20°C until further analysis.

Blood chemistry measurements

Routine laboratory methods was used to measure the levels of blood glucose, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, total triglycerides (TG), and serum C-reactive protein (CRP). A thorough description of these methods can be found in the literature.[13]

TIC assay

To estimate serum trypsin inhibitory capacity (TIC), enzymatic tests was conducted.[6] The serum antitryptic proteins suppressed BAPNA hydrolysis by trypsin in Tris buffer. Serum dilution process was performed for 100 times in 100 mM Tris buffer with a pH of 8.2 at 37°C, followed by mixing with diluted trypsin solution. Afterward, the obtained solution was blended with trypsin synthetic substrate (BAPNA) and incubated for 5 minutes at 37°C. Bovine serum albumin was regarded as control. A spectrophotometer was used to measure the absorbance value of each sample, and TIC was determined.

Analysis of plasma AAT and determination of AAT phenotype

The analyses of AAT phenotypes was performed using isoelectric focusing (IEF) gel electrophoresis in an ampholine solution with a pH of 4.2-4.9 in accordance with the previously described methods.[13] After applying the samples to a 0.1% agarose IEF gel, the electrophoresis was performed for 40 min (2,000 V at 20°C). To visualize AAT bands, peroxidase-conjugated AAT-antiserum was used, so that after each addition of acidic dimethylformamide to hydrogen peroxide, a pigment was produced. Moreover, to determine the AAT phenotype, visual inspections were performed and compared with known patterns.

Statistical analysis

Data analysis was performed using SPSS software V21 (SAS Institute, Cary, NC, USA). Kolmogorov–Smirnov test was applied to examine the normality of data distribution. To examine group-wise differences, unpaired Student's t-test was used. The Mann-Whitney U-test was employed where normality of the data was rejected. Frequency calculations were performed using Fisher's exact test. Tests with a P value less than 0.05 were regarded as statically significant and the data were demonstrated as mean ± standard deviation or median.

Results

Diabetic patients showed higher values of body mass index (BMI) in comparison with control group, however, no significant difference in age, sex, height and weight was observed between the two groups [Table 1]. Compared with control subjects, the diabetic group had lower plasma levels of HDL ($P < 0.05$), but higher plasma levels of CRP ($P < 0.05$), glucose ($P < 0.05$), and LDL-C: HDL-C ratio ($P < 0.05$). The results of independent t-test indicated that patients with T1DM had lower activity of plasma AAT compared with the control group. Also, there was no significant difference in the level of plasma AAT and sex between the two groups. The serum TIC level of patients with T1DM (2.35 ± 0.34 μmol/min/ml) was significantly lower than normal group (3.36 ± 0.36 μmol/min/ml) ($P < 0.001$). The frequency of phenotype MM in healthy subjects was 100%; and

in patients with T1DM, the frequency of phenotype MM, MS and MZ was 61.9%, 23.8% and 14.3%, respectively ($P < 0.001$). The mean level of TIC in T1DM individuals had the lowest and highest frequency in MZ and MM phenotypes, respectively. One-way ANOVA showed that the association between the type of AAT phenotype and its activity in patients with T1DM was not statistically significant ($P > 0.05$). The analysis also showed that this relationship is not statistically significant in terms of gender, age group and duration of T1DM ($P > 0.05$). Table 2 compares the distribution of AAT phenotype in T1DM patients and healthy subjects. Also, Tukey's multiple comparisons test showed that the mean duration of T1DM in patients with MZ phenotype was significantly higher than patients with MM and MS phenotypes ($P < 0.05$), while did not differ significantly with MM and MS phenotype y ($P = 0.438$). This is a notable finding, that T1DM diagnosis appears to occur earlier in MS/MZ individuals than MM. Figure 1 shows the pattern of electrophoresis associated with healthy subjects and patients with T1DM.

Table 1: Patient demographics and measured plasma markers

Variable	Diabetics (*n*=42)	Controls (*n*=42)	*P*
Sex (male/female)	21/21	21/21	1.000
Age (mean±SD, years)	23.55±3.18	23.57±3.17	0.973
Weight (kg)	68.55±9.38	66.69±8.74	0.351
Height (cm)	167.67±7.96	171.24±8.60	0.052
BMI (mean±SD, kg/m2)	24.43±3.34	22.72±2.24	0.007
AAT (mean±SD, mg/ml)	1.09±0.37	1.21±0.28	0.420
Cholesterol [mean±SD, mmol/l]	5.9±0.9	6.1±1.01	0.096
HDL (mean±SD, mmol/l)	1.07±1.37	1.26±0.96	0.002
LDL (mean±SD, mmol/l)	5.06±0.31	5.67±0.82	0.643
LDL: HDL ratio [median (range)]	3.28 (1.10-8.37)	3.97 (1.00-9.29)	0.042
TG [median (range), mmol/l]	2.12 (0.17-8.25)	1.70 (0.25-6.12)	0.057
CRP [median (range), mg/l]	0.32 (0.05-4.65)	0.19 (0.01-2.89)	0.001
Glucose [median (range), mmol/l]	12.26 (5.78-32.14)	5.63 (4.16-6.97)	0.012

AAT, α1-antitrypsin; BMI, body mass index; LDL, low-density lipoprotein; HDL, high density lipoprotein; TG, triglyceride

Table 2: Comparison of the distribution of AAT phenotype in patients with T1DM and healthy subjects

Phenotype AAT	Diabetics (*n*=42) (%)	Controls (*n*=42) (%)	*P*
Women			
MS	7 (33.3)	0	<0.001
MM	11 (52.4)	21 (100)	
MZ	3 (14.3)	0	
Men			
MS	3 (14.3)	0	
MM	15 (71.4)	21 (100)	<0.021
MZ	3 (14.3)	0	
Age 18-23 year			
MS	6 (30)	0	
MM	9 (45)	19 (100)	<0.001
MZ	5 (25)	0	
Age 23-28 year			
MS	4 (18.2)	0	
MM	17 (77.3)	23 (100)	<0.022
MZ	1 (4.5)	0	
Total			
MS	10 (23.8)	0	
MM	26 (61.9)	42 (100)	<0.001
MZ	6 (14.3)	0	

Discussion

In this study, the levels of serum TIC, AAT and phenotypes were analyzed in plasma samples. In addition we determined the serum glucose, and lipid profile in diagnosed patients with T1DM and healthy participants. We report that the activity of plasma AAT and the serum TIC level of patients with T1DM was significantly lower than healthy participants. The frequency of phenotype MM in healthy subjects was 100%; and the frequency of phenotype MM, MS and MZ in T1DM patients, was 61.9%, 23.8% and 14.3%, respectively.

Recent investigations have shown abnormal plasma AAT levels in T1DM subjects. Similar to our study, other study have demonstrated that the total AAT inhibitory activity, as well as, plasma AAT concentration were lower in T1DM subjects than in control subjects.[14] Moreover, recent studies have indicated that the levels of s-TIC are lower in patients with Type 1 and 2 diabetes mellitus compared to controls; there is an inverce relationship between TIC levels and diabetes length ($r = -0.5420$, $P < 0.0001$).[6] The study by Park SS *et al.* demonstrated the potential role of AAT therapy as an anti-inflammatory agent in the prevention of type 1 diabetes, which was consistent with the results of this study.[10] Kalis *et al.*[15] showed that AAT increases insulin secretion and also protects β-cells against apoptosis-inducing cytokines. According to the results of their study, it can be concluded that AAT can be used as a therapeutic method to increase insulin secretion, which is consistent with our study, which indicates a lack of AAT activity in people with type 1 diabetes.

The activity of proteolytic enzymes may be affected by human plasma inhibitors.[16] The present study explored the possible variations of s-TIC in patients with diabetes mellitus. The results of our study showed a decrease in s-TIC in diabetic

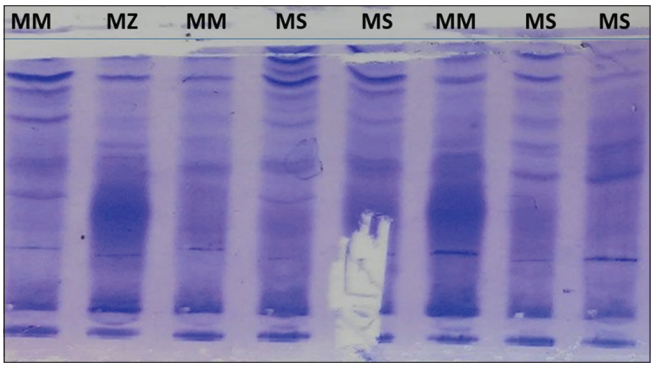

Figure 1: The pattern of isoelectric focusing (IEF) gel electrophoresis associated with healthy subjects and patients with T1DM

patients, which may be attributed to the nonenzymatic glycosylation of AAT. Lysine is the most probable amino acid that undergoes glycosylation. When present in a polypeptite chain, lysine includes a free amino group. The important role of lysyl residues in AAT physiological function was indicated in experiments that treated AAT with maleic anhydride and acetic anhydride.[6] In the presence of proteinase inhibitors such as AAT, undesirable proteolysis happens and proper protection against tissue damage would not be possible. Decreased function of proteinase inhibitors due to glycosylation may explain some of the complications of diabetes, including eyes, kidneys, and other organs.[17]

There are several genetic variants of AAT associated with decreased plasma levels of AAT. A combination of S-, Z- and the null alleles are mostly responsible for intermediate-to-severe AAT insufficiency phenotypes. According to the recent epidemiologic studies, there are at least 3.4 million deficiency allele combinations (SS, ZZ and Pi SZ) and 116 million carriers (MS and Pi MZ) all over the world.[18] There is compelling evidence indicating that the development of severe ZZ AAT insufficiency is associated with increased risk of neonatal hepatitis, liver cirrhosis, chronic obstructive pulmonary disease, and hepatocellular carcinoma.[19] The levels of plasma AAT in people heterozygous for Z or S allele (MS or MZ) are 55–80% of that in normal people.[20] Up to now, no association has been found between these intermediate AAT deficiency genotypes and the development of specific disease. In the present study, AAT alleles were determined in 42 patients with T1DM, and the frequency of phenotype MZ, MS and MM was 14.3%, 23.8% and 61.9%, respectively. It was while all the healthy participants in control group had MM AAT. Finally, additional clinical studies are recommended to assess AAT genotype in patients with T1DM and evaluate the rate of β-cell apoptosis in diabetic patients having low to normal AAT levels.

Considering the importance of diabetes, in order to achieve a definitive therapeutic approach and find a relationship between AAT genotypes and T1DM, it can be suggested that future studies should be done by recruiting a larger statistical population. Also, markers involved in inflammation and diabetes should be investigated and other methods of AAT measurement and molecular mechanisms are also used. In conclusion, the results showed a decrease in trypsin inhibitory capacity and AAT activity in people with T1DM. Therefore, AAT can be used as a clinical marker, given the fact that AAT insufficiency has many negative effects on tissues and organs, it can be used as a pharmaceutical agent to treat the development and complications of T1DM.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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