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Impaired Activity of Serum Alpha 1-protease Inhibitor in Diabetes Mellitus

[1,2]M. Naderi, [2,3]M. Hashemi and [3]S. Ghavami
[1]Department of Infectious Diseases and Tropical Medicine, School of Medicine,
Zahedan University of Medical Sciences, Zahedan, Iran
[2]Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital,
Zahedan University of Medical Sciences, Zahedan, Iran
[3]Department of Clinical Biochemistry, School of Medicine,
Zahedan University of Medical Sciences, Zahedan, Iran

Abstract: Alpha-1-antitrypsin (AAT) is the prototypic member of the serine protease inhibitor (serpin) superfamily of proteins, which have a major role in inactivating neutrophil elastase and other proteases to maintain protease-antiprotease balance. In this study the serum AAT was measured using enzymatic assay in diabetic patients. The serum trypsin inhibitory capacity (s-TIC) was determined in 144 outpatients with uncontrolled insulin-dependent diabetes mellitus (n = 39) and non insulin dependent diabetes mellitus (n = 104). The s-TIC values were 1.960±0.399, 2.002±0.4304 and 2.867±0.395 μmol min^{-1} mL^{-1} in IDDM, NIDDM and healthy subjects, respectively. The diabetics groups had a significantly lower s-TIC in their serum than did the healthy control ($p<0.0001$). We found that there is a negative correlation between s-TIC and the duration of diabetes disease ($r = -0.5420$; $p<0.0001$). There was no correlation between S-TIC and age of patients ($p = 0.865$). We found that in diabetic patients, glucose dysregulation have significant effects on the trypsin inhibitory capacity of plasma.

Key words: Serum trypsin inhibitory capacity, s-TIC, diabetes, IDDM, NIDDM

Introduction

Alpha-1-antitrypsin (AT) deficiency was first described in the late 1960s in patients with severe pulmonary emphysema. The recognition of AT deficiency as a cause of emphysema then led to what is still the prevailing theory for the pathogenesis of emphysema, the protease-antiprotease theory (Perlmutter, 2004). Alpha-1-antitrypsin is the principal serum protease inhibitor. Levels of alpha-1-antitrypsin (alpha1-AT), the key protease inhibitor, are genetically determined by alleles that present in many phenotypes, some of which are associated with deficiency of the protein. Alpha-1-antitrypsin is called serine protease inhibitor (SERPIN) which complexes with the active site of serine proteases, thus blocking their enzyme activity. AAT has a single polypeptide chain of 394 amino acid residues with three carbohydrate side chains, giving a total molecular weight of 51 KD. Its small size allows AAT to pass into all body fluids and inhibits most serine protease, especially those related to trypsin. Although there is some local tissue synthesis (e.g., in monocytes and macrophages), nearly all plasma AAT is synthesized by the hepatic parenchymal cells (Ghavami *et al*., 2005; Hashemi *et al*., 2005).

Glycosylation as a non-enzymatic unregulated process is observed to be taking place in condition of hyperglycemia. Accelerated non-enzymatic glycosylation of many body proteins altering their function. The aim of this study was investigated possible alterations in serum α1 antitrypsin activity in diabetic patients.

Corresponding Author: Dr. Mohammad Naderi, Department of Infectious Diseases and Tropical Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

Materials and Methods

Materials

Trypsin, α-N-benzyl-DL-arginine-p-nitroaniline (BAPNA), Bovine Serum Albumin (BSA) were purchased from sigma. Tris, $CaCl_2$, HCl, DMSO and acetic acid were analytical grade.

Patients

This study was performed since May 2004 until April 2005 in clinic of diabetes, Bou-Ali Hospital, Zahedan, Iran.

Blood samples were obtained from uncontrolled IDDM (n = 47; 9 male, 38 female, age range 14-75 years), uncontrolled NIDDM (n = 97; 30 male, 67 female, age range 30-80 years) and healthy control (n = 51; 24 male, 27 female, age range 11-66 years). The serum was separated off within 2 h of collection of the samples and stored at -20 °C until analyzed.

s-TIC Aassay

Serum-Trypsin Inhibitory Capacity (s-TIC) was measured using enzymatic assay (Dietz *et al*., 1974). The antitryptic proteins of serum inhibit the hydrolysis of α-N-benzoyl-DL-arginine-*p*-nitoanilide (BAPNA) by trypsin in Tris buffer. Briefly serum was diluted 100 times with 100 µM Tris buffer (pH = 8.2 at 37°C) and then mixed with diluted trypsin solution. These solutions were then mixed with trypsin synthetic substrate (BAPNA) and incubated at 37°C for 5 min (bovine serum albumin was considered as a control). The absorbance value for each sample was read by a spectrophotometer at 400 nm and s-TIC was calculated (Ghavami *et al*., 2005; Hashemi *et al*., 2005).

Results

Figure 1 shows the s-TIC of uncontrolled IDDM (1.960±0.399 µmol min^{-1} mL^{-1}), NIDDM (2.002±0.4304 µmol min^{-1} mL^{-1}) and healthy control (2.867±0.395 µmol min^{-1} mL^{-1}) subjects. In diabetic patients the s-TIC was significantly ($p<0.0001$) lower than that of healthy control. Diabetic patient was found to have decreased inhibitory capacity towards the proteolytic enzyme trypsin.

As shown in Fig. 2 the s-TIC of diabetic male (n = 39; s-TIC = 1.987±0.439 µmol min^{-1} mL^{-1}) and female (n = 105; s-TIC = 2.038±0.418 µmol min^{-1} mL^{-1}) was not statistically significant ($p = 0.77$).

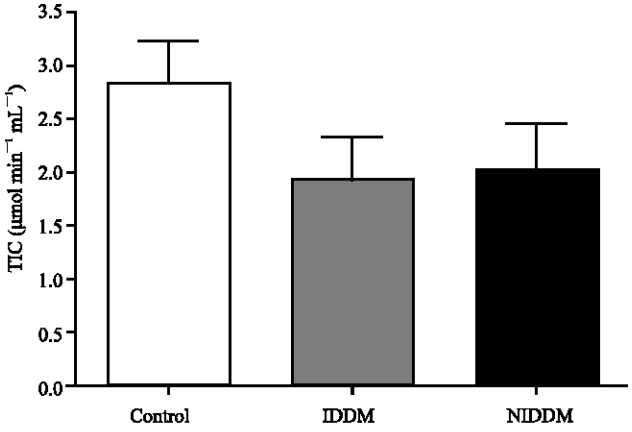

Fig. 1: Comparison of serum trypsin inhibitory capacity (s-TIC) in healthy subject (control), IDDM and NIDDM

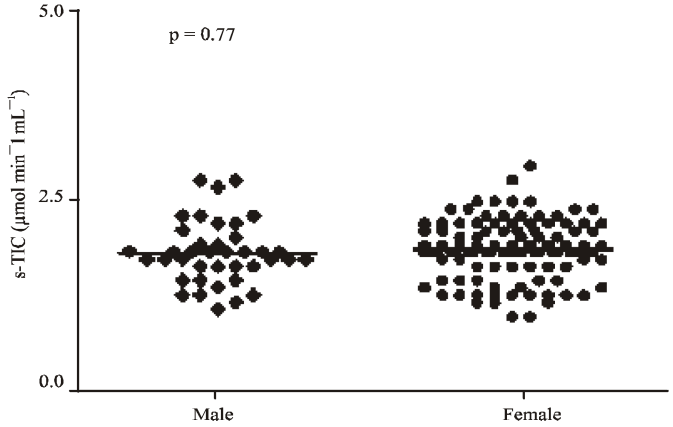

Fig. 2: Comparison of s-TIC in male and female diabetic patients

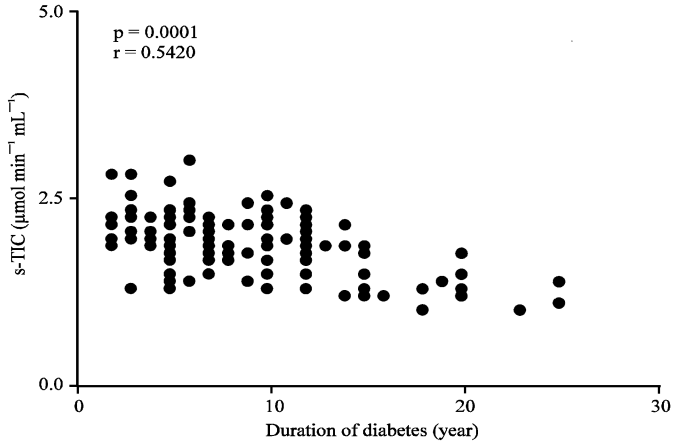

Fig. 3: Correlation between s-TIC and duration of diabetes

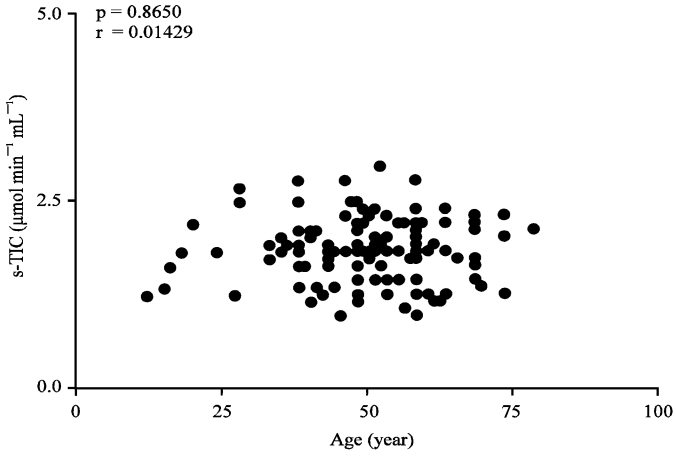

Fig. 4: Correlation between s-TIC and age in diabetic patient

Figure 3 shows the correlation between s-TIC (n = 144; 1.100-3.200 μmol min^{-1} mL^{-1}) and duration of diabetes (n = 144; 2-25 years). The s-TIC decreases significantly with increasing duration time of diabetes disease ($r = -0.5420$; $p<0.0001$).

As shown in Fig. 4 there is no correlation between age of diabetic patients and s-TIC ($r = 0.01429$; $p = 0.8650$).

Discussion

The term nonenzymatic glycosylation represents the interaction of glucose with specific free amino groups in proteins under mild physiological conditions not catalyzed by specific enzymes. Poor short-term glycemic control was associated with higher elastase concentration in plasma and neutrophils and measurements of plasma polymorphonuclear neutrophil elastase level can be considered as a marker of development of diabetic angiopathy (Piwowar *et al*., 2000). It has been reported that the concentration of α_1 antitrypsin have increase in diabetic patients (Ganrot *et al*., 1967), but the serum trypsin inhibitory capacity was decrease in diabetic patients (Phadke *et al*., 1998). The trypsin inhibitory capacity of α_2 macroglubulin has been reported to be decreased in condition of diabetes mellitus (Roberts *et al*., 1986). Decrease in cardiac contractility is a hallmark of chronic diabetes advanced glycation end products (AGEs) are formed on intracellular ryanodine receptor calcium-release channel (RyR2) during diabetes (Bidasee *et al*., 2003). Nonenzymatic glycosylation of plasma proteins may contribute to the excess risk of developing atherosclerosis in patients with diabetes mellitus (Duell *et al*., 1990).

Human plasma contains inhibitors, which control the activity of proteolytic enzymes. Alpha-1-proteinase inhibitor (α_1 antitrypsin) and α_2 macroglobulin are two of them present in high concentration in human plasma, which inhibit action of trypsin among other proteinases (Phadke *et al*., 1998). In the present study we investigated possible alterations in serum trypsin inhibitory capacity in diabetic patients. We found that the serum trypsin inhibitory capacity (s-TIC) of plasma decreases in diabetes patients. The mechanism of decrease in TIC is due to nonenzymatic glycosylation of α_1 antitrypsin. The most probable amino acid undergoing glycosylation is Lysine which has free amino group when present in a polypeptite chain. Importance of lysyl residues for the physiological function of α_1 antitrypsin was reported in studies when α_1 antitrypsin was treated with maleic anhydride or acetic anhydride which react with lysyl residues specifically (Travis and Salvesen, 1983). If proteinase inhibitors like α_1 antitrypsin alter their normal function, limiting of unwanted proteolysis and prevention of tissue damage cannot be achieved properly. Glycosylation of proteinase inhibitors to decrease their function may partly explain some of the complications of diabetes involving eyes, kidneys and other organs that cannot be prevented by meticulous control of glycaemia (Phadke *et al*., 1998).

In conclusion, present results showed that in uncontrolled diabetic patients, glycosylation of α_1 antitrypsin reduce trypsin inhibitory capacity.

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