Mast cell, a new player in type 2 diabetes

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Background
Mast cells plays critical role in inflammatory diseases, including cardiovascular and metabolic diseases and their associated complications. These cells exert their physiological and pathological activities by releasing granules containing histamine, cytokines, chemokines, and proteases, including mast cell-specific chymases and tryptases
Aim of our study is to detect the role of mast cell in diabetic obese and its correlation to different diabetic complications
Method
70 Type 2 diabetic obese patients attending the Diabetes and Endocrinology clinic in Kasr El Ani hospital compared to 15 healthy controls
All patients were subjected to: Full medical history, complete physical examination, Anthropometric measurements (BMI, waist circumference) fasting glucose (assessed after 8 hours fasting) –A1C – serum cholesterol –triglycerides-LDL-HDL(assessed after 12 hours fasting) Tryptase level
Results
Statistical significant difference between patients and control regarding BMI, glucose,cholesterol, HDL, LDL,tryptase ($p<0.001$) ,triglycerides($p=0.001$)
Tryptase statistically correlated with BMI,fastingglucose,A1C,triglycerides ($p=0.014$,$r=0.031$)/($p=0.012$,$r=0.297$)/($p<0.001$,$r=0.862$),($p=0.039$,$r=0.247$)
Higher mean level of tryptase in patients with diabetic complications mainly in retinopathy (32.32 ng/ml)
Conclusion
Tryptase participate in the pathogenesis of diabetes mellitus and its complication targeting mast cells as novel therapy for diabetes requires further investigations

Keywords:
type 2 diabetes, mast cells, tryptase

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Introduction

Diabetes is a metabolic disorder characterized by hyperglycemia and associated with microvascular and macrovascular syndromes mediated by mast cells (MCs). MCs are activated through cross-linking of their surface high-affinity receptors for immunoglobulin E (IgE) (FcεRI) or other antigens, leading to degranulation and release of stored inflammatory mediators, and cytokines/chemokines without degranulation. MCs are implicated in innate and acquired immunity, inflammation, and metabolic disorders such as diabetes. Histamine and tryptase genes in MCs are overexpressed in pancreatic tissue of type 2 diabetes mellitus (T2DM) patients [1].

MCs are essential components of asthma and allergic responses; recent studies have shown that these cells are important in diet-induced obesity and T2DM [2].

MCs are critical effectors in inflammatory diseases, including cardiovascular and metabolic diseases and their associated complications. These cells exert their physiological and pathological activities by releasing granules containing histamine, cytokines, chemokines, and proteases, including MC-specific chymases and tryptases [3].

One of the best-known mechanisms of MC activation is the binding of IgE to its high-affinity receptor FcεRI on the MC surface. After IgE binding, MCs release histamine, MC protease, proteoglycan, cytokines, and chemokines [4], all of which participate in metabolic diseases [5,6].

Two types of MCs, differing in neutral (cytoplasmic) proteases, have been identified: MCs that contain tryptase and MCs that contain tryptase and chymase [7].

Tryptase is a trypsin-like serine proteinase that has been estimated to constitute ~20% of the total cellular protein of human MCs. This is stored fully active in the

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cytoplasmic granules of all human MCs and is released in the peripheral circulation [8].

The aim of the study is to detect the role of MC in diabetic obese and correlate it to different diabetic complications.

Patients and methods

In a cross-sectional study, we studied 70 type 2 diabetic obese patients attending the Diabetes and Endocrinology Clinic in Kasr El Ainy hospital and 20 healthy controls. The participants were recruited after approval of the institutional ethical committee.

All patients were subjected to the following:

A full assessment of medical history including drug history, complete physical examination, neurological examination, administration of the Michigan Neuropathy Screening Instrument for the detection of peripheral neuropathy score, and anthropometric measurements (BMI, waist circumference). The exclusion criteria were malignant disease, chronic liver disease, or kidney failure.

All patients were subjected to fundus examination, ECG, and echocardiography.

A written consent was obtained from all participants of this study after an explanation of the study was provided.

Laboratory investigations

These included fasting glucose (assessed after 8 h of fasting), A1C, serum cholesterol, triglycerides – low-density lipoprotein (LDL)–high-density lipoprotein (HDL) (assessed after 12 h fasting), and tryptase level.

Four milliliters of fasting (12 1 h) venous blood samples were withdrawn from each participant in the study and divided into two parts: the first part was placed in an EDTA-containing tube for the determination of glycosylated hemoglobin by cation exchange resin [9]. The second part was allowed to clot and centrifuged at 3000*g* for 10 min, and the separated serum was stored at −20°C for the determination of fasting blood glucose, total cholesterol, triglyceride, HDL, LDL, and tryptase.

The determination of fasting blood glucose, total cholesterol, triglyceride, HDL, and LDL was carried out on Hitachi 912 (Roche Diagnostics GmbH, Manheim, PA, USA) using colorimetric techniques.

Serum tryptase was determined using a sandwich enzyme immunoassay supplied by EIAab (Biopark, Optics Valley, Wuhan, China) [10].

Statistical analysis

The statistical analysis for the social sciences, 10.0 using the statistical package SPSS version 15 (SPSS Inc., Chicago, Illinois, USA) for Windows was used for data management and analysis, and the Microsoft power point for charts. Quantitative data were presented as mean±SD. For comparison of the two groups means, Student's *t* test was used, whereas for the comparison of the means of the three groups, one-way analysis of variance was used, followed by a post-hoc test. A *P* value was considered significant at 0.05.

Results

A total of 70 type 2 diabetic patients were studied; the mean age of the study group was 49.26±9.34 years and the duration of diabetes was 11.2±3.2 years. There were 51 women and 19 men. A total of 24 patients were on oral hypoglycemic agents, 34 patients were receiving treatment with insulin, and 12 patients were receiving insulin and oral hypoglycemic agents.

The characteristics of the studied groups are shown in Table 1; there was a statistically significant difference between patients and controls in BMI, glucose,

Table 1 Characteristics of patients and controls in the study

	Patients		Control		*P* value
	Mean	SD	Mean	SD	
BMI	35.67	4.63	23.87	2.07	<0.001
Waist (cm)	117.60	11.16	–	–	
Fasting glucose (mg/dl)	230.44	77.72	82.73	8.23	<0.001
A1C	8.52	1.54	4.53	.73	<0.001
Cholesterol (mg/dl)	218.89	19.57	171.87	24.44	<0.001
TG (mg/dl)	118.97	13.75	106.47	10.13	0.001
HDL (mg/dl)	37.85	6.80	54.25	5.42	<0.001
LDL (mg/dl)	154.01	18.33	129.00	5.46	<0.001
Tryptase	37.05	7.93	7.49	1.79	<0.001

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

Table 2 Correlation of tryptase with different parameters

	Tryptase
Age	
r	−0.046
P value	0.707
N	70
BMI	
r	0.031
P value	0.014
N	70
Waist	
r	0.042
P value	0.731
N	70
Duration	
r	−0.057
P value	0.638
N	70
Fasting glucose (mg/dl)	
r	0.297
P value	0.012
N	70
A1C	
r	0.862
P value	<0.001
N	70
Cholesterol (mg/dl)	
r	−0.136
P value	0.262
N	70
TG (mg/dl)	
r	−0.247
P value	0.039
N	70
HDL (mg/dl)	
r	−0.132
P value	0.276
N	70
LDL (mg/dl)	
r	0.068
P value	0.578
N	70

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

cholesterol, HDL, LDL, tryptase ($P<0.001$), and triglycerides ($P=0.001$).

Tryptase correlated with BMI, fasting glucose, A1C, and triglycerides, and this was statistically significant ($P=0.014$, $r=0.031$)/($P=0.012$, $r=0.297$)/($P<0.001$, $r=0.862$)/($P=0.039$, $r=0.247$), as shown in Table 2 and Figs 1 and 2.

Tryptase in relation to complications

A higher level of tryptase was found in diabetic patients with complication either micro vascular or microvascular.

In patients with retinopathy (four patients)tryptasec level wasv 38.2 ng/ml,in patients with peripheral neuropathy (six patients) trptase level was level 35.1 ng/ml, in diabetic with ischemic heart disease (eight patients) tryptase level 37 ng/ml,in diabetic patients with cerebrovascular stroke (three patients) tryptase level level 35.4 ng/ml.

Discussion

Different studies in experimental animals and humans have suggested the role of MCs in obesity and diabetes [11].

However, the study of tryptase levels in type 2 diabetes in humans and its correlation to complication is limited.

Serum MC tryptase levels were shown to be significantly higher in obese patients than in lean patients, suggesting a role of these inflammatory cells in obesity and diabetes [12,13].

Chymase and tryptase may play a direct or indirect role in the development of obesity.

Liu and colleagues reported that MCs play a direct role in T2DM.

DIO mice also developed glucose intolerance and insulin resistance, which did not develop in *Kit*W-sh/W-sh and *Kit*W/Wv MC-deficient mice.

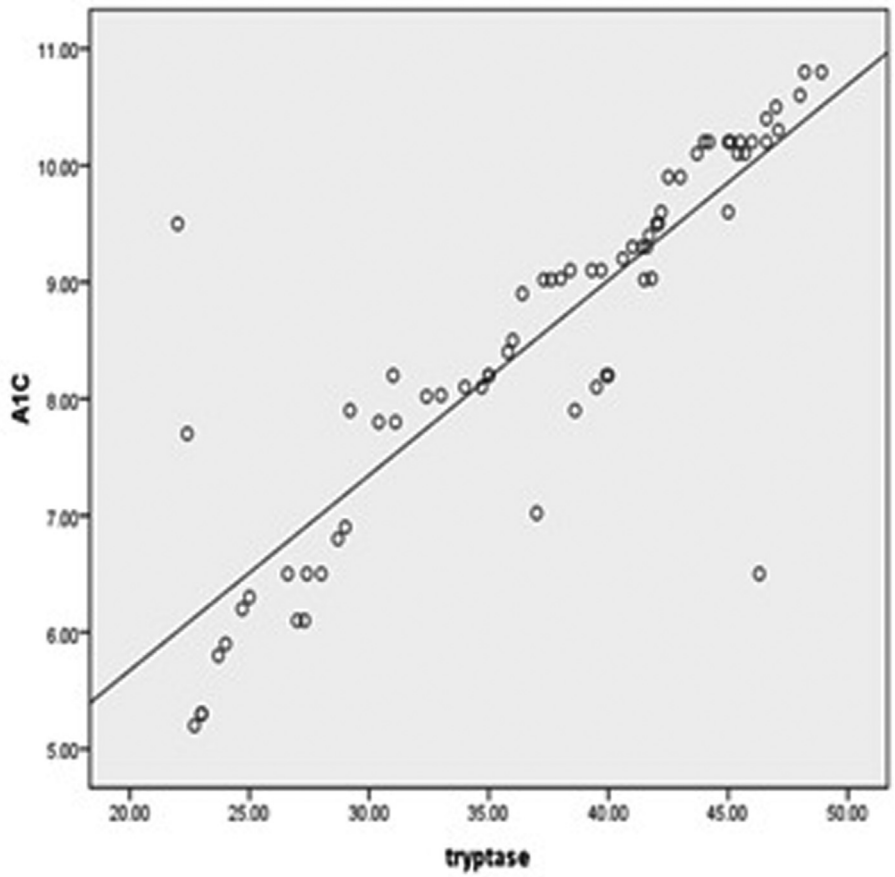
Diabetic mice had high serum insulin and glucose levels and high KIT-positive MCs in white adipose tissue [12].

In our study, there were statistical significant differences between patients and controls in BMI, glucose, cholesterol, HDL, LDL, tryptase ($P<0.001$), and triglycerides ($P=0.001$).

Wang *et al.* [13] found that plasma tryptase levels were also higher in T2DM patients than in those with prediabetes or with normal glucose levels.

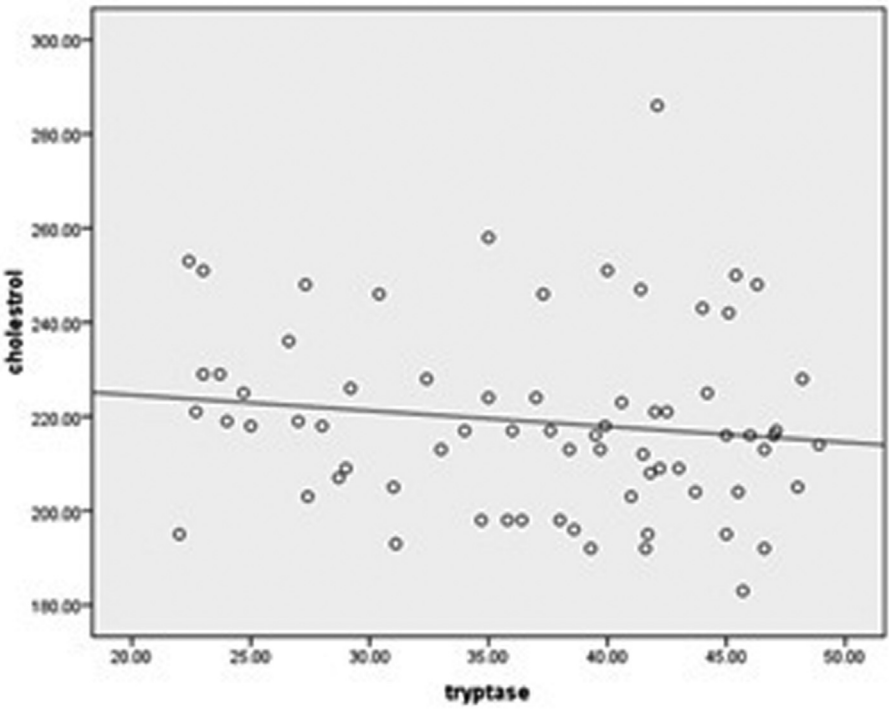
In a study carried out by Aina 2013 involving 80 patients with confirmed diabetes, 189 patients with prediabetes, and 71 normal controls, chymase and IgE levels in the blood were significantly higher in diabetic patients, followed by those with prediabetes. In an ordinal logistic model, interactions between IgE and chymase markedly increased the risk [odds ratio (OR)] of developing diabetes before [OR, 2.479 (1.079–5.778), $P=0.033$] and after [OR, 2.594 (1.118–6.018), $P=0.026$] adjustment for age, sex,

Figure 1



Correlation between tryptase and A1C.

Figure 2



Correlations between tryptase and cholesterol.

hypertension, waist circumference, waist-to-hip ratio, BMI, total cholesterol, triglyceride, HDL, LDL, hyperinsulinemia, and homeostatic model assessment indexes. Although not statistically significant, interactions between IgE and tryptase also increased the risk of developing diabetes by 2.091 (P=0.068) and 2.167 (P=0.057) folds before and after the same adjustment. These data suggest that MCs and MC chymase and tryptase are potential drug targets for human diabetes or prediabetes [3].

In our study, in terms of the correlation of tryptase with different variables, it was found that tryptase correlated with BMI, fasting glucose, A1C, and triglycerides, which is statistically significant (P=0.014, r=0.031)/(P=0.012, r=0.297)/(P<0.001, r=0.862), (P=0.039, r=0.247).

MCs, which localize to various organs including the lungs, heart, and kidneys [5], also play a central role in the pathogenesis of different diabetic complications.

In terms of diabetic nephropathy, chymase participates in the conversion of pro-MMP-9 into MMP-9, a protease implicated in diabetic nephropathy and diabetic retinopathy, and this increase is associated with glomerulosclerosis, tubulointerstitial fibrosis, and vascular fibrosis in patients with diabetic nephropathy [5].

Tryptase promotes the production of vascular endothelial growth factor, which is implicated in the development of diabetic nephropathy [14–16].

In our study, a higher mean tryptase level was found in patients with complications (36.32 U), both microvascular or macrovascular complications.

In the past few years, accumulating evidence has established the contribution of the MC toward cardiovascular diseases as well, in particular, its effects on atherosclerotic plaque progression and destabilization. Through its release not only of mediators, such as the MC-specific proteases chymase and tryptase, but also of growth factors, histamine, and chemokines, activated MCs can have detrimental effects on their immediate surroundings in the vessel wall. This results in matrix degradation, apoptosis, and increased recruitment of inflammatory cells, thereby actively contributing toward cardiovascular diseases [17].

In terms of the role of therapy in treatment MC-related diseases. Wang and colleagues studied two types of MC-deficient mice, along with corresponding wild-type control mice, that were fed a western diet to induce obesity and diabetes. They also used two anti-allergy drugs, cromolyn, and ketotifen (Zaditor), to treat wild-type mice during intake of a western diet or after the onset of obesity and diabetes, to examine the possible prevention or reversal of these conditions.

The administration of cromolyn or ketotifen with a western diet or a regular chow diet yielded much greater reductions in body weight gain and glucose tolerance compared with the diet change alone, suggesting a role of MC inactivation in reversing obesity and diabetes [5].

Mice deficient in cathepsin L or cathepsin K, or treated with their selective inhibitors, are leaner than control mice or show significantly improved glucose sensitivity are fully protected from developing T2DM [12].

MC deficiency or pharmacological stabilization reduced body weight gain and improved glucose and insulin sensitivities. These common, side effect-free drugs also reduced pre-established obesity and diabetes without noticeable toxicity. Mechanistic studies suggest that MCs participate in these metabolic disorders by affecting energy expenditure, protease expression, angiogenesis, apoptosis, and preadipocyte differentiation [13].

Some diabetic patients may benefit from enhancing Mast Cell survival and proliferation this requires detailed basic researches and clinical studies [11].

Histamine and tryptase genes in MCs are overexpressed in pancreatic tissue of T2DM patients. Histamine is a classic inflammatory mediator generated by activated receptors of MCs from the histamine-forming enzyme histidine decarboxylase, which can be activated by two inflammatory chemokines, RANTES and MPC1, when injected intramuscularly or intradermally in mice [1].

In a patient with T2DM, less than 6 months of treatment with cromolyn reduced both plasma glucose and glycosylated hemoglobin levels to normal ranges [18].

In a similar model of diabetes induced by a single high dose of streptozotocin (60 mg/kg), hamsters showed high blood glucose levels, increased pancreatic chymase, and Ang-II forming activities. Mice receiving TY-51469, a chymase inhibitor, showed significantly reduced blood glucose, chymase, total Ang-II forming activities, malondialdehyde, and MC numbers in pancreatic tissues compared with those receiving placebo treatment. Furthermore, the

TY-51469 group had significantly more pancreatic islets than the placebo group [19].

A significant improvement in the controls in blood glucose and kidney complications after treatment with different chymase inhibitors suggests a direct involvement of this MC protease – and possibly tryptase – in the pathogenesis of diabetes. Targeting these MC proteases may become a powerful means of managing metabolic diseases and their complications.

Drug companies may be awaiting more mechanistic studies from chymase-deficient or tryptase-deficient animals as these inhibitors may have off-target effects. For example, chymase inhibitor RO5066852 also targeted cathepsin G, although IC50 is 27-fold higher than chymase [17]. Better results in chymase inhibitor protease-deficient animals may help to advance the progress of proposing human trials.

Molecular targets on MCs for novel therapies have promising therapeutic possibilities on the basis of preclinical studies [20,21].

We hope that in the near future, human trials will emerge to examine the efficacy of MC stabilizers and MC protease inhibitors in obesity, diabetes, and associated metabolic complications.

However, further studies are needed to clarify the role of MCs in prediabetes and diabetes.

Conclusion

Tryptase participates in the pathogenesis of diabetes mellitus and its complications, although direct evidence is currently not available, a hypothesis that merits further investigation for novel therapies targeting MCs.

Limitation of the study

The small number of diabetic patients included in our study is a limitation and further future studies are required to establish the correlation between tryptase and diabetic complications including nephropathy, which was not tested in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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