

# STATUS OF CIRCULATING SERUM TRYPTASE IN HEALTHY OBESE AND COMORBIDLY OBESE CONDITIONS

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## ABSTRACT

### *Background*

Serum Tryptase is one of most abundant protease secreted by mast cells and it account as indirect measure for a number of human abnormalities, such as mastocytosis, systemic anaphylaxis, obesity, allergy, asthma and various forms of cancer.

### *Objectives*

In this study we focused on obese individuals and we investigated the relationships between total Serum Tryptase with age, gender, classes of obesity with/or without type 2 diabetes mellitus or dyslipidemia.

### *Methods*

Estimation of serum tryptase, body mass index, lipid profile, and serum glucose were performed in population of 250 participants aged 12-80 years.

### *Results*

Serum tryptase significantly increased with body mass index, with higher level of serum tryptase found among diabetic obese than healthy obese individuals. There was significantly increase serum tryptase with age, but no correlation was found between serum tryptase and gender, hemoglobin A1c, and fasting blood glucose.

### *Conclusion*

Level of serum tryptase increases with age, being much more pronounced in adults older than > 16 years old than individuals < 16 years old regardless of their body mass index. Serum tryptase is significantly higher in serum's of all obese participants compared to the healthy participants, regardless of the obesity-related-complications; while the obesity related type 2 diabetes mellitus patients shows a significantly highest level of serum tryptase compared to all other groups.

**Keywords:** *Serum Tryptase, obesity, type 2 diabetes mellitus, dyslipidemia, body mass index*

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## INTRODUCTION

Tryptase is the most abundant protein produced by human mast cells. It is a neutral serine protease and it is loaded as granules inside mast cells. The granules of tryptase are released from mast cells when they are activated. Therefore, tryptase could be considered as a marker of mast cell activation or mast cell burden<sup>(1)</sup>. In humans, tryptase protein has four isoforms, but only two of them; namely alpha ( $\alpha$ ) and beta ( $\beta$ ), are medically important and their protein sequences have nearly 90% sequence identity. The other types are gamma ( $\gamma$ ), and delta ( $\delta$ ) have no medical significances<sup>(2)</sup>. The  $\beta$ -tryptase is a predominant form of tryptase stored in mast cell granules and high levels can be seen during severe inflammation such as systemic anaphylaxis<sup>(3)</sup>. Tryptase has been suggested to involve in different biological processes by cleavage of different substrate through protease receptors (PARs) that leads to varying effects/responses<sup>(4)</sup>.

Nowadays, total serum tryptase is increasingly used as a signature for various abnormalities in clinical practice. Tryptase level in biological fluid indicates the body burden of mast cells and are useful as biomarker of clonal mast cell disorders, mastocytosis. Tryptase is also a useful marker for a differential diagnosis of systemic anaphylaxis<sup>(3)</sup>. Individuals who are allergic to hymenoptera venom, increase level of tryptase in them indicate severe reactions<sup>(5)</sup>. It is known that  $\beta$ -tryptase is also over expressed in asthma. This suggests mast cell involvement in late asthmatic response and in chronic inflammation<sup>(6)</sup>. It is also a potential marker for some hematological conditions such as hypereosinophilic syndrome (HES)<sup>(7)</sup>, and myeloid neoplasm<sup>(8)</sup>. Although studies reveals a positive association between obesity and increase number of mast cells in white adipose tissue of both animals and humans, the exact mechanism of mast cell in pathogenesis of obesity is not fully understood<sup>(9)</sup>. There are limited evidences to confirm the relation between obesity and serum tryptase<sup>(6,10)</sup>. However, the association between serum tryptase and obese diabetics is not fully understood. Previous works show a direct contribution of mast cells in type 2 diabetes mellitus (T2DM) and insulin resistance in animal models<sup>(9)</sup>. Another study shows a positive correlation between mast cells and obese diabetics. It also shows that mast cells in these patients tend to release more inflammatory cytokines than obese non-diabetic patients<sup>(11)</sup>.

## MATERIALS AND METHODS

### Study Cohort

This study involves a total of 250 participants, samples from 150 individuals collected in central laboratory of Sulaimani city, and 100 samples collected from patients visited the center of diabetes and endocrine glands. The tests were done in the research laboratory in the medical school. The Ethics Committee of School of Medicine approved the study and written informed consent has been obtained from all involved participants. The participants were divided into four groups: Group I; Includes one hundred patients out of 250 participants were obese based on their body mass index (BMI) equal and more than 30kg/m<sup>2</sup> with type 2 diabetes mellitus (T2DM). Group II; Includes seventy-eight patients out of 250 were obese with dyslipidaemia. Group III; includes twenty-two obese individuals without obesity related complications. Group IV; includes fifty individuals out 250 participants who were normal weight control healthy volunteers based on their body mass index (BMI between 18.5 to 24.9kg/m<sup>2</sup>).

### Sample collection

Ten milliliters of venous blood was drawn from each individual cubital vein using disposable syringes. Five milliliters blood have been collected in plain tubes and allowed for 20 minutes at room temperature to clot. Serum were separated by centrifugation at 3000 rpm for approximately 20 minutes, also 5 milliliter of blood collected in EDTA tubes, and both tubes stored at -20°C in the medical research laboratory at school of medicine until assayed.

### Measurements of biochemical markers

Total serum tryptase was measured by enzyme linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay human mast cell tryptase. Measurement of lipid profile and fasting blood glucose and HbA1c was measured by Cobas automated analyzer systems.

### Measurement of body mass index

Measurements of body height and weight were done for all individuals in light clothing without shoes, and with empty bladder. Weight was measured by calibrated physician scale and height by a sliding bar attached to the scale. BMI was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>), and all individuals in this study were classified by world health organization as normal weight and obese

according to their BMI <sup>(12)</sup>, obese individuals were also subdivided into class 1 (BMI 30-34.9 kg/m<sup>2</sup>), class 11 (BMI 35-39.9 kg/m<sup>2</sup>), class 111 (BMI ≥ 40 kg/m<sup>2</sup>) obesity based on their BMI.

**Statistical analysis**

ANOVA and Chi-Squared test were used to compare tryptase concentrations between all subgroups. Independent sample t-test was used to show the association between serum tryptase, HbA1c and fasting blood glucose. P-value less than 0.05 were regard as statistically significant.

**RESULTS AND DISCUSSIONS**

The Study Group and Characteristics: In this work, we have studied the level of Serum Tryptase (ST) in a random selection of 250 participants, including healthy individuals. The level of Serum Tryptase increased significantly with age (Table 1) and it was statistically significant (p<0.05). Conversely, no significant association between ST was found among males and females in the study groups. Grouping of the samples were based on their body mass index (BMI). Among them 80% were obese regardless of their status for related-diseases and/or obesity-related-complication and 20% are healthy normal weight (healthy control group) group based on their BMI, personal and family history information.

**Correlation between ST and Lipid Profiles**

Among different biochemical markers of dyslipidaemia, serum low density lipoproteins (LDL) and high density lipoproteins (HDL) were statistically associated with ST, while serum total cholesterol, triglyceride (TG) and serum very low density lipoproteins (VLDL) did not have any associated with ST (Figure 3 and 4). Serum Tryptase is increasing with increasing bodyweight regardless of their health status and the levels were statistically significant regarding to association between BMI and serum tryptase parameters (Figure 3). Moreover, a statistically significant difference was found between means of serum tryptase in classes of obesity (Table 2).

On-the-other-hand, higher level of ST was found among patients with type 2 diabetes mellitus (T2DM) compared to healthy obese individuals (Figure 1). We looked the comparison between Serum Tryptase levels among controls (HbA1c 4.5-7%) and uncontrolled (HbA1c >7%) in T2DM group. Then, we found non-significant differences regarding hemoglobin A1c (HbA1c) level between controlled and uncontrolled T2DM (Figure 1). In addition, we could not find any relation between ST and fasting blood glucose (FBG) (Figure 2).

**Table 1. ANOVA test between mean serum tryptase concentrations in relation to age classes.**

Serum Tryptase in different age group				
Age(years)	Number	ST (Mean±SD)	SE	P-value
12-30	55	4.39±2.65	0.36	
31-40	49	6.72 ± 2.75	0.39	
41-50	65	9.18±3.60	0.45	<0.005
51-60	55	9.86±3.74	0.50	
61-70	20	9.16±3.84	0.86	
>70	6	7.91±4.95	2.02	

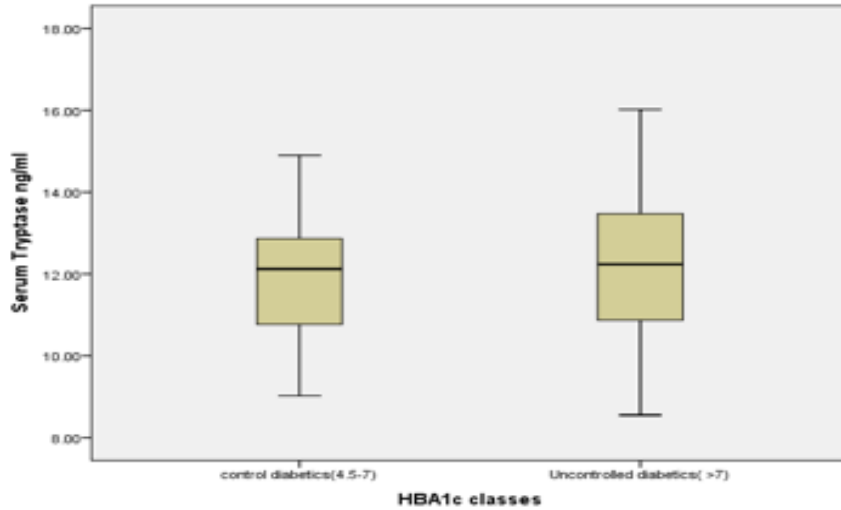


Figure 1. Serum Tryptase concentration according to HbA1c in diabetic patients.

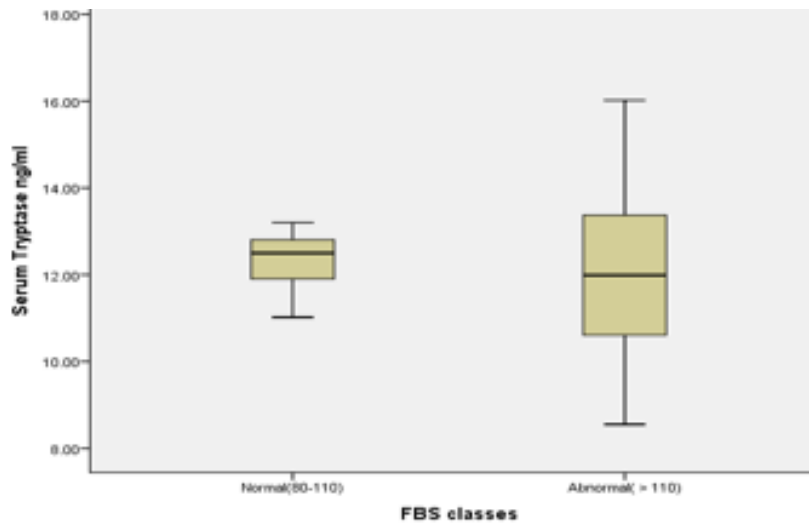


Figure 2. Box-Whisker plot between normal and abnormal FBG and serum tryptase among diabetics.

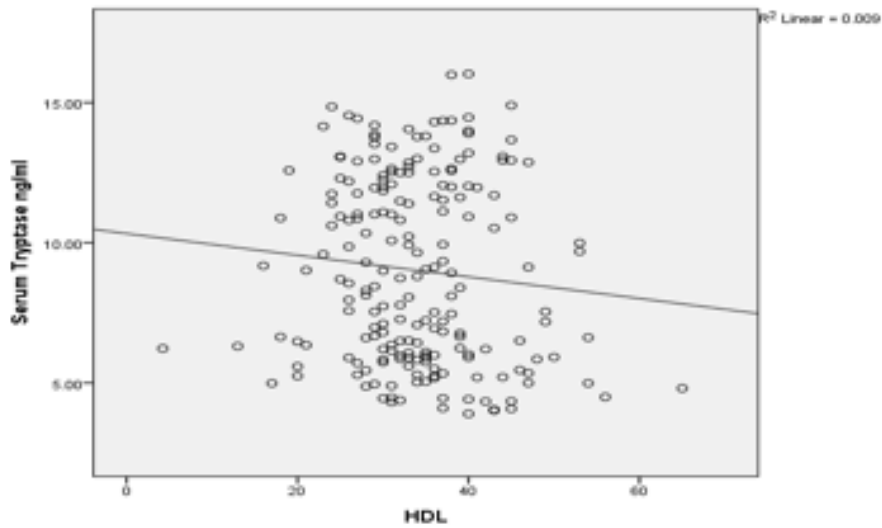


Figure 3. Linear regression analysis shows a negative correlation between serum tryptase in concentration and S.HDL.

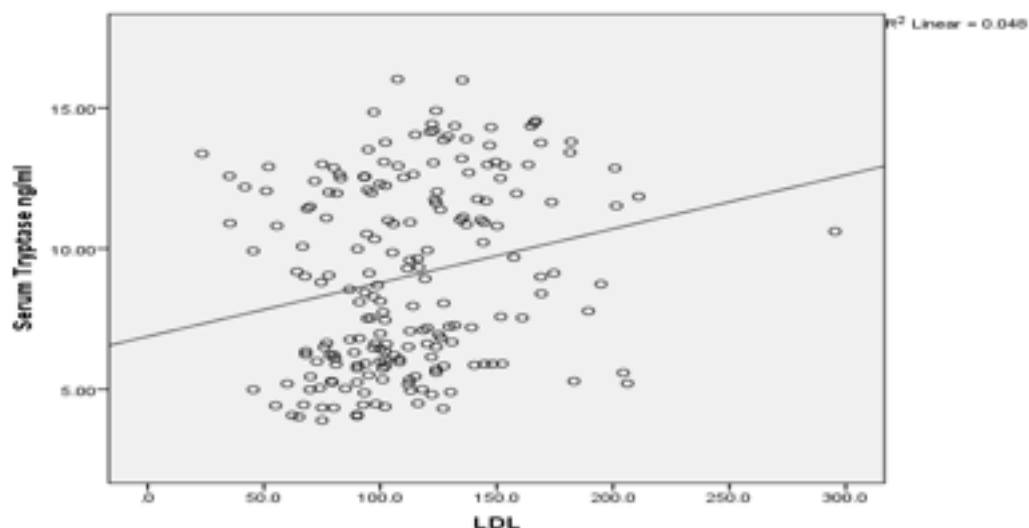


Figure 4. Linear regression analysis shows a positive correlation between serum tryptase concentration and S.LDL.

Table 2. ANOVA comparisons between mean serum tryptase in all three classes of obesity.

Mean of Serum Tryptase in relation to classes of obesity				
Classes of obesity	Number	ST (Mean ± SD)	SE	P-value
Class I obesity	139	9.00 ±3.29	0.27	
Class II obesity	41	8.52 ±2.98	0.46	< 0.001
Class III obesity	20	10.11 ±3.98	0.89	

### BMI in correlation to ST

In the present study body-fat composition was reflected by BMI and it was found that significantly associated with ST. This was independent of possible confounding factors. As indicated, a significant variance in level of ST among obese participants and normal weight control group were found in sulaimani city. Possible explanation for the association between ST and obesity is that white adipose tissue in obese individuals is more abundant and contains more mast cell numbers than the lean counterparts. However, the exact mechanisms of mast cell in the pathogenesis of obesity are not fully understood. Previous studies did a staining of human WAT section with mast cell tryptase monoclonal antibody and they found a higher number of mast cells in WAT from obese tissues compared with WAT from lean subjects<sup>(13)</sup>. Since higher ST is reflecting the mast cell burden or mast cell activity, this may explain why ST is higher in obese than normal weight humans<sup>(14)</sup>. At the same time a present study showed that there is a

significantly increasing level of ST in all the classes of obesity with highest level in class III obesity than class II, and class I obesity.

### Obese with T2DM complications

Regarding obese patients with type 2 diabetes mellitus previous investigations show that BMI, absolute weight gain, and abdominal adiposity all are independent risk factors for diabetes<sup>(15)</sup>. Although family history and age are accepted risk factors<sup>(16)</sup>, only weight change is controllable<sup>(17)</sup>.

In the current study, the measurement of ST shows that there is a significant elevation in obese patients with T2 DM compared to obese non-diabetic group. The exact role of ST in the Pathophysiology of T2DM has not yet been fully understood in humans<sup>(9)</sup>. In mouse model, it is believed to be a direct contribution of mast cell in insulin resistance and T2DM developments. It has been demonstrated genetically that mast cell deficient mice has higher insulin sensitivity and glucose tolerance and

reduces body weight compared to wild-type mice <sup>(9)</sup>. Divoux et al have found that mast cell was increased in T2DM humans, and the secretion of tryptase was increased in obese participants especially obese diabetic patients <sup>(11)</sup>.

#### **HbA1c in correlation to ST**

Regarding HbA1c in present study there was no significant association between ST in controlled and uncontrolled diabetes based on their HbA1c level (Figure 2). In similar fashion, there was no significant association between ST and fasting blood glucose (FBG) in diabetic patients (Figure 3). However, a previous work shows a positive correlation between ST and HbA1c and FBG <sup>(11)</sup>. This difference in the result between this current and the previous study could have originated from different variables, which could not be resolved in a single study. Other confirmatory research studies needed to resolve this issue. A number of factors that may contribute in these variations are cultural background, geographical changes and the life style.

#### **Dyslipidemia correlation with ST**

The current study shows a significantly higher level of ST among obese patients with dyslipidemia than obesity without complication but slightly lower ST than obese diabetic groups. The significant, positive association between ST and dyslipidemia could point to an important contribution of mast cells in obese dyslipidemic patients that may induce subclinical atherosclerosis. A previous work by Moreno et al. <sup>(18)</sup> show that mast cells are involved in the development of atherosclerosis by releasing cytokines that induce mast cell protease expression specially circulating ST. This has been proven as the true relation between ST and carotid intima-media thickness (c-IMT) and they concluded that ST has proatherogenic role in subclinical atherosclerosis.

Among dyslipidemic parameters, high serum triglyceride (TG) level  $\geq 160$ mg/dl is found in 43.6% of our obese patients, while normal TG  $< 160$ mg/dl is found in 36.4% of patients. This shows that most of the obese patients have elevated TG than normal TG. This phenomenon has been shown in previous studies <sup>(19)</sup>. Since both TG and ST increase in obese patients, we examined the relation between ST and TG among obese participants, but no association was found between them among our participants. This finding is consistent with a previous study <sup>(6)</sup>. Similar to TG, the present

study didn't find significant association between serum cholesterol, VLDL and ST among obese participants, in consistent with previous study <sup>(6)</sup>.

Conversely, in the current study there was a significant ( $P < 0.041$ ) association between ST and LDL found among other markers of lipid profile, with a positive relation. This result is in disagreement with the previous study done by Fenger, showing that mast cell activation does not influence serum LDL in humans and another study shows that no association on mice <sup>(20)</sup> as like in humans. What could contribute to these differences is the assay method for either LDL, which is not accurate, or the data, which depends on lifestyle. On the other hand, the data in the present study are consistent with other studies done on mice that show activation of mast cells rise LDL level <sup>(21)</sup>.

It is well known that accumulation of LDL particle in the intima (the inner layer of arterial wall) is taken up by macrophage and leads to the formation of foam cells. In addition to macrophage, mast cells also present in the arterial intima and leads to stabilization of foam cells <sup>(11)</sup>. Activation of mast cells increases LDL uptake by macrophage this will stimulates IgE-dependent sensitized mast cell and release of its component such as neutral proteases, including ST and histamine <sup>(13)</sup>. Therefore, this may explain how the relation between ST and LDL is regulated.

Unlike LDL, HDL, in the current study, demonstrates the inverse relation with ST. The data show a statistically significant negative association between ST and HDL. This result is consistent with recent studies, showing also similar association between ST and HDL <sup>(6)</sup>. This inverse relation between ST and HDL could be due to the finding that mast cells degrade apolipoproteins on HDL (ApoE, ApoA-I, ApoA-IV), thereby causing a functional change in HDL by decreasing its ability to remove cholesterol from arterial intima back to the blood stream <sup>(22)</sup>.

#### **Conclusions**

In this study, we aimed to evaluate the serum tryptase levels in obese individuals in our locality. This attempt was to find any correlation between activities of mast cells in obese population, by measuring serum tryptase. According to the results obtained we reached to the conclusions: (1) The level of ST increases with age, being much more pronounced in adults older than  $> 16$  years old than individuals  $< 16$  years old regardless of their BMI. (2) The ST concentration does not differ

between male and female participants. (3) The ST is significantly higher in serum's of all obese participants regardless of their obesity-related-complications compared to the healthy participants. (4) The obesity related T2DM shows a significantly higher level of ST than obese individuals without complications while FBG and HbA1c status have no significant association with ST concentration. (5) The dyslipidemia related to obesity demonstrates a higher ST than healthy obese individuals. Among dyslipidemia markers, only LDL and HDL show a significant association between ST, with LDL showing a positive relation, while HDL showing a significantly inverse relation with ST. Collectively, the ST level alterations could reflect the pathophysiological status of the obese condition, including the obese-related-complications like T2DM and dyslipidemia as a pro-inflammatory mediator.

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