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Correlation between Serum Tryptase Level and Disease Severity in Asthmatic Patients in the Sulaimani Governorate

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ABSTRACT

Asthma is a chronic inflammatory disorder of the lower airways, characterized clinically by reversible airway obstruction and bronchial hyper-responsiveness (BHR). Tryptase is a neutral serine protease and is the most abundant mediator stored in mast cell granules. The release of tryptase from the secretory granules is a characteristic feature of mast cell degranulation in allergic diseases. This study aims to evaluate the difference between serum tryptase levels and asthma severity in the Sulaimani governorate. Five milliliters of venous blood were obtained from 85 healthy control and 85 asthmatic patients were subdivided into three groups; controlled asthma (n = 25), partly controlled asthma (n = 30) and uncontrolled asthma (n = 30). Serum tryptase level was analyzed by using sandwich ELISA. There was a significant difference among the study groups, with highest serum tryptase levels in the uncontrolled asthma group (7.68 ± 0.65 ng/dl) and lowest levels in the controlled asthma group (4.4 ± 0.15 ng/dl). Serum tryptase levels increase significantly with age. There was a significant difference between ex-smokers and never-smokers among asthmatic subjects. No significance in serum tryptase levels was found between males and females and between asthmatic subjects with negative and positive family history of asthma. Serum tryptase levels in uncontrolled asthma were significantly higher than in controlled asthma. Serum tryptase levels are elevated with age and also in ex-smoker asthmatic subjects. However, there was no significant difference in the levels of serum tryptase according to gender and family history of asthma.

Keywords: asthma, severity, allergy, serum typtase, ex-smoker, ELISA

INTRODUCTION

Asthma affects more than 300 million individuals worldwide. It is one of the most common chronic diseases and its global incidence continues to increase. Historically, the increase was assumed to occur predominantly in industrial countries, but recent studies show that asthma is now predominant in developing countries [1, 2].

Mast cells have long been deemed to play an important role in the pathophysiology of asthma through their capability to release a host of pleiotropic autacoid mediators, proteases, and cytokines in response to activation by both immunoglobulin E-dependent and diverse non-immunologic stimuli[3]

Tryptase is a neutral serine protease with trypsin-like activity. It is one of the major protein components of mast cells in higher eukaryotes [4]. Mast cells appear to express α-, β-, and γ-tryptases. The main types stored in their

secretory granules. However, the β-isoenzymes accumulate in much larger amounts than any of the other granule-associated serine proteinases of leukocytes and lymphocytes, comprising as much as 25% of the mast cell protein. For that reason, β-tryptases also are the major isoenzymes that are released during mast cell degranulation and that can be isolated from normal human lung and skin tissues [5]. Because β-tryptase is resistant to inhibition by biologic inhibitors of serine proteases such as α1-proteinase inhibitor, α2-macroglobulin, and aprotinin, regulation of its activity may depend on regulating its association with heparin proteoglycan [6]. The biological activities of enzymatically active tryptase are not clear from the involvement of mast cells in diseases like mastocytosis, anaphylaxis, urticaria, and asthma. The most relevant biological substrate(s) of tryptase is still uncertain, even though several potential contenders have been evaluated, primarily *in vitro*. β-Tryptase can cleave many proteins and its predicted biological outcomes may include anticoagulation, kinin generation and destruction, fibrosis and fibrolysis, enhancement of vasopermeability, cell surface protease-activated receptor (PAR)-2 activation, angiogenesis, inflammation, and airway smooth muscle hyper-reactivity. Revealing the significance of these potential activities *in vivo* remains a challenge [7].

The aim of this study is to examine elevation of serum tryptase levels in asthmatic patients and also to evaluate the difference between serum tryptase levels and disease severity in asthmatic patients in Sulaimani governorate.

MATERIALS AND METHODS

The samples for the study include 145 subjects (72 males and 73 females), including 60 control individuals. All of the recruited individuals gave their informed consent before they were engaged in the study. Eighty-five of the participants are known and already diagnosed asthma cases. The samples collection started from February to August 2014. Sixty samples were collected from healthy controls at the Central Medical Laboratory and eighty-five asthmatic patients mainly at The Center of Asthma and Allergy in Sulaimani Governorate. The Ethics Committee at the School of Medicine, University of Sulaimani, approved the study project.

The eighty-five asthmatic patients are divided into three groups according to severity by GINA guidelines[8].

- **Group 1:** Twenty-five samples were patients with controlled asthma.
- **Group 2:** Thirty samples were patients with partly controlled asthma.
- **Group 3:** Thirty samples were patients with uncontrolled asthma.

Sample Collection: Using disposable syringes, five milliliters of venous blood were obtained from participants in the study mainly from the arm through the Cubital vein. The drawn blood was then stored in a plain tube and left at room temperature for 25 minutes to clot. It was then centrifuged for 15 minutes at 3500 rpm to separate the serum and was kept frozen at (-65)^{o}C until they were assayed.

Measurement of Mast cell tryptase (MCT): The human mast cell tryptase was measured using enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Mast Cell Tryptase.

Pulmonary Function Tests (PFT): The primary instrument used in pulmonary function testing is the spirometer. It is designed to measure changes in volume and can only measure lung volume compartments that exchange gas with the atmosphere.

Statistical Analysis: All the results were expressed as mean ± standard deviation (SD). The data were analyzed by using GraphPad Prism 6.01 software (Graph Pad Software Inc, San Diego, CA, USA). Unpaired *t*-test and one-way ANOVA followed by Tukey's post hoc test were utilized for statistical evaluation of the differences between the means.

RESULTS

Serum Tryptase Level and Asthma: The serum tryptase level was measured in this study for asthmatic patients and control individuals. The results were (4.385 ± 1.248 ng/ml) and (6.025 ± 2.929 ng/ml) in normal control and asthmatic patients respectively. These results clearly indicated significant differences ($p < 0.05$) in the serum tryptase level of control and asthmatic patients (Figure 1).

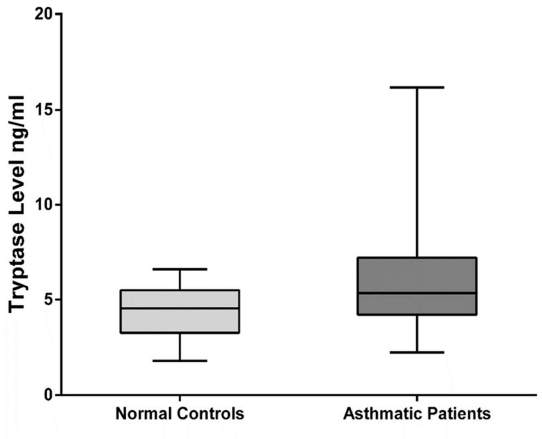

Figure 1: Box-Whiskers plot showing the difference in serum tryptase level between all asthmatics patients and normal control

Serum Tryptase Levels in all Groups: ANOVA test was carried out for the comparison of serum tryptase levels among asthma groups (controlled, partly controlled and uncontrolled) and normal controls. The results obtained for serum tryptase were (4.3 ± 1.2 ng/ml) in the normal control, (4.4 ± 1.5 ng/ml) in the controlled asthma group, (5.7 ± 2.2 ng/ml) in the partly controlled asthma group and (7.6 ± 3.5 ng/ml) in the uncontrolled asthma group. P-value was < 0.05, indicating significant differences between control and case groups for serum tryptase (Figure 2), also confirmed by Tukey's post hoc test.

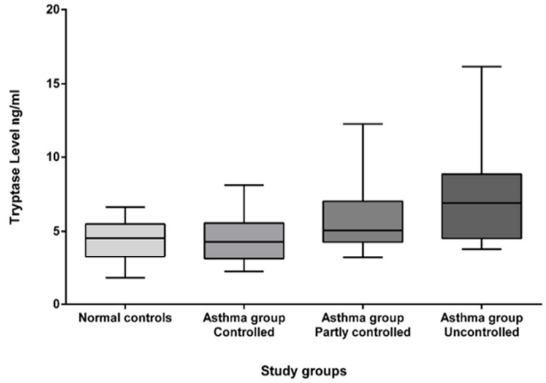

Figure 2: Box-whiskers plot of serum tryptase concentration across study groups

Table 1: Tukey's multiple comparison test showing mean difference (Mean Diff.), 95% confidence intervals of difference (CI of Diff.) and significance between each group

Tukey's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significance
Normal vs. Controlled Asthma group	-0.026	-1.36 - 1.31	No
Normal vs. Partly controlled Asthma group	-1.3	-2.59 - -0.07	Yes
Normal vs. Uncontrolled Asthma group	-3.2	-4.55 - -2.03	Very highly
Controlled Asthma group vs. Partly controlled Asthma group	-1.3	-2.83 - 0.22	No
Controlled Asthma group vs. Uncontrolled Asthma group	-3.2	-4.79 - -1.74	Very highly
Partly controlled Asthma group vs. Uncontrolled Asthma group	-1.9	-3.42 - -0.51	Highly

Multiple Comparisons of Serum Tryptase Levels between Each Group: Tukey's multiple comparisons test was done to find significance between study groups. The results in Table 1 indicate that there are no significant differences between normal and the controlled asthma group or between the controlled and partly controlled asthma groups. There were significant differences between normal and the partly controlled asthma group and highly significant differences between the partly controlled asthma and uncontrolled asthma group while differences were

found to be very highly significant between normal and the uncontrolled asthma group and between the controlled asthma and uncontrolled asthma groups.

Gender Distribution of the Cases: The total number of blood samples from asthma patients was 85, with42 from males and 43 from females. There is no statistically significant difference between serum tryptase level and gender unpaired t-test and the p-value is more than 0.05 (Figure 3).

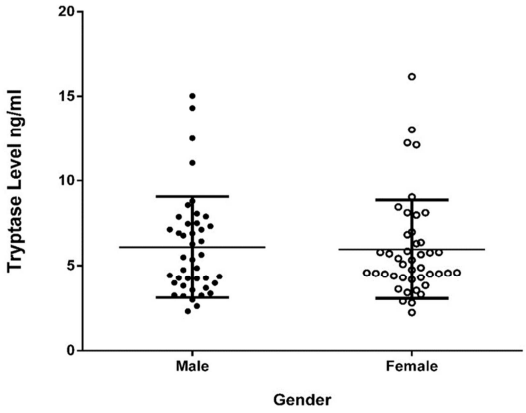

Figure 3: Scatter plot of serum tryptase levels and gender

Age Distribution of the Cases: The ages of 85 asthmatic patients ranged from 5 to 24, 25 to 44 and over 45 years. ANOVA and post hoc tests were used for different age groups and the results showed a p-value of less than 0.05, which in turn means that serum tryptase level increases with age (Figure 4). Linear regression also showed a positive correlation between age and serum tryptase levels with $R^2 = 0.114$ (Figure 5).

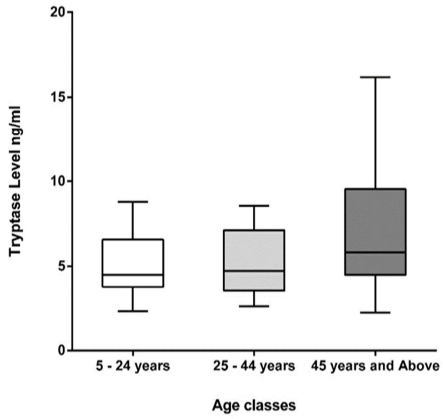

Figure 4: Box-whiskers plot of age distribution of asthmatic patients

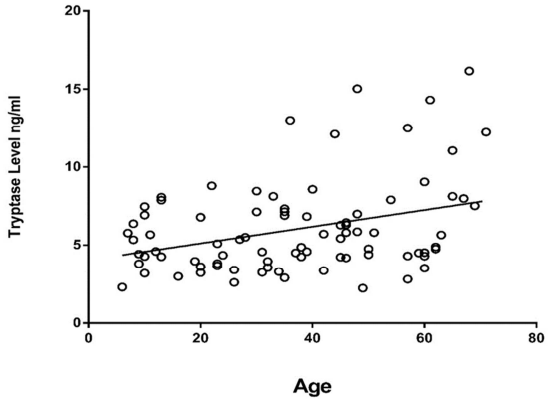

Figure 5: Linear regression shows a positive correlation between serum tryptase levels and age in years (R^2 = 0.114)

Serum Tryptase and Family History of Asthma: Of the 85 cases, the asthmatic patients with negative family history were 48 and asthmatic patients with positive family history were 37. There is no significant difference in tryptase levels between the asthmatic patients with negative family history and asthmatic patients with positive family history (Figure 6).

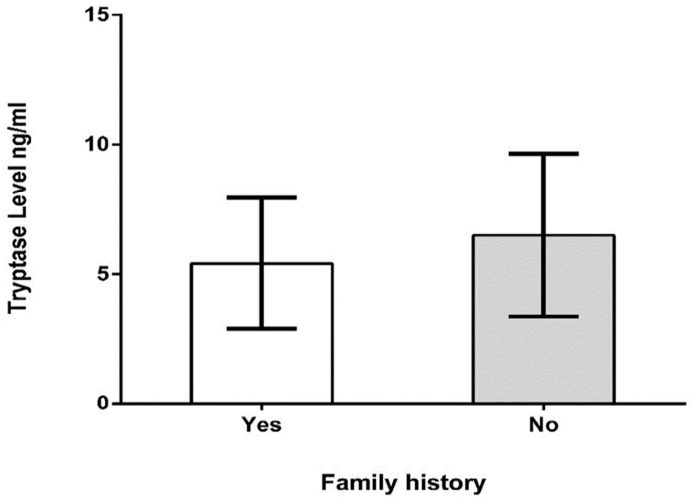

Figure 6: Column bar graph shows the difference in serum tryptase levels between negative and positive family history groups in asthmatic patients

Serum Tryptase Level and Smoking Status in Asthmatic Patients: Smoking status is divided into two groups: those patients whom never smoked (71 cases) and those that are ex-smokers (14 cases), since there are no current smokers among the participants. There was a significant difference in serum tryptase levels between never-smokers and ex-smokers of asthmatic patients (Figure 7).

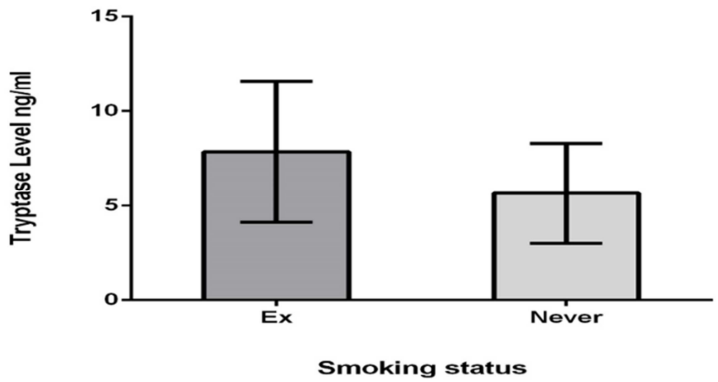

Figure 7: Column bar graph shows the difference in serum tryptase levels between none and ex-smokers

DISCUSSION

Serum Tryptase Levels in Asthma: Serum tryptase was chosen for the present study because it is a prominent constituent of, and specific to, the mast cell and is relatively more stable than histamine in serum. It is considered to be a reliable indicator of mast cell degranulation [9]. Tryptase levels are increased in the lungs of asthmatics and tryptase inhibitors have been shown to have important effects in reducing both early and late-phase airway responses [10-12]. In the present study asthma severity (classified according to GINA guidelines by WHO) found significant differences in serum tryptase levels and also a significant difference was confirmed between the levels of asthma severity and the normal population ($P < 0.05$) (Figure 1). This implicates that serum tryptase levels increase with an increase in disease severity from controlled to partly controlled and uncontrolled asthma in Sulaimani governorate (Figure 2,Table 1). Similar studies conducted show that severe asthma is associated with a predominance of tryptase and chymase positive mast cells (MC_{TC}) with increased expression of FcεRI and surface-bound IgE in the airway submucosa and epithelium [13, 14]. Therefore, the activity of mast cells increases with the increase in the severity of asthma, which is mainly caused by an ongoing inflammation in alveolar tissue, leading to elevated levels of serum tryptase in asthmatic patients.

Serum Tryptase Levels and Gender in Asthma: Among 85 samples in this study, 42 were males and 43 were females. The result shows no statistical difference in serum tryptase levels between males and females (P value > 0.5), but the mean serum tryptase levels in male subjects was slightly higher than in female subjects (Figure 3). The correlation between gender and basal serum tryptase levels is still controversial. Despite the fact that there is no significant difference between male and female basal tryptase levels, some studies show that males have higher basal tryptase levels than females [15-19]. Nevertheless, other studies show that the mean of basal tryptase level in female subjects is slightly higher than in male subjects [20, 21]. The influence of gender could be on the total body burden of mast cells, the production and processing of tryptase by mast cells, or the metabolism of spontaneously secreted tryptase[21]. The effect of gender on the clinical use of total tryptase levels in serum requires further thorough assessment, but should be considered when interpreting test results.

Serum Tryptase Levels and Age: All participants in this study were divided into three age classes: 5-24 years, 25-44 years and 45 years and above. The result show a significant difference between serum tryptase levels and age (P value < 0.01). Therefore, the data reveal that serum tryptase levels increases with age (Figure 4 and 5). Although it is well known that serum tryptase levels correlate positively with age[22]. A study by Sahiner (2014) shows that in the paediatric age group higher levels of serum tryptase can be found during infancy age 0-1 year.It also shows that basal tryptase levels in children are similar to those in adults [15]. A study by Tsukioka (2010) indicates that asthma severity is also significantly associated with age at onset in patients with adult-onset asthma [23]. This means that the serum tryptase level can be used as a good marker for asthma and can be considered as a risk factor for asthma severity in older individuals.

Serum Tryptase Levels and Family History of Asthma: Family history is introduced as a strong risk factor for asthma in adults and childhood asthma [24-28]. Moreover, studies confirmed that a greater asthma severity is associated with a positive family history of asthma [25, 29, 30]. Among 85 cases of asthma involved in the study, 48

cases had a negative family history, whereas 37 cases were found to have a positive family history with either first or second degree relatives. The result demonstrate no significant difference between family history and serum tryptase levels (Figure 6). This outcome is also confirmed by Gasiorowska (2006) clarifying that family history of allergy does not correlate with serum tryptase concentrations [31].

Serum Tryptase Levels and Smoking Status in Asthmatic Patients: Cigarette smoking is associated with accelerated decline of lung function, increased mortality, and worsening of symptoms in asthma. Moreover, exposure to cigarette smoke can alter the inflammatory mechanisms in asthma to become similar to that seen in COPD, with increasing CD8 cells and neutrophils, and may therefore alter the response to therapy. Cigarette smoke exposure has been associated with a poor response to inhaled corticosteroids, which are recommended as first line anti-inflammatory medications in asthma [32]. A significant difference is found between serum tryptase levels and smoking status (Figure 7). This result shows a significant difference between never-smoker and ex-smoker asthmatic patients with a higher mean tryptase level in ex-smokers than never-smokers. There were no current smokers among the study individuals. A study by Small-Howard (2005) shows that cigarette smoking up-regulates mast cell-secreted proteinases levels in mast cells at both the protein and the mRNA level [33]. Another study by Kalenderian (1988) demonstrates that smokers have elevated histamine and tryptase levels in broncho-alveolar lavage fluid, suggesting increased mast cell degranulation [34]. Ex-smokers show persistent mucosal inflammation similar to that seen in current smokers [35, 36]. Therefore, it is expected to observe a higher serum tryptase levels in ex-smokers than never-smokers in asthmatic patients. Furthermore, this study suggests that smoking history is preferable to be taken into consideration before measuring serum tryptase level in asthmatic patients.

CONCLUSION

Serum tryptase levels are significantly increased with asthma severity. Specifically, serum levels of tryptase are higher in individuals with uncontrolled and partly controlled asthma than controlled asthma and normal population. There is no significant difference in serum tryptase levels in males and females. Older patients showed significantly higher serum tryptase levels. Family history of asthma does not affect serum tryptase concentration in asthmatic patients. Ex-smokers have higher serum tryptase levels, suggesting that smoking directly influence stryptase concentrations as it activates mast cells and induces more inflammation.

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