ORIGINAL ARTICLE

Basal Serum Tryptase Level Correlates With Severity of Hymenoptera Sting and Age

I Kucharewicz,[1] A Bodzenta-Lukaszyk,[1] W Szymanski,[1] B Mroczko,[2] M Szmitkowski[2]

[1] Department of Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland
[2] Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok, Poland

Abstract

Background: Increased serum tryptase has been linked to the severity of the reaction after Hymenoptera stings. The aim of the study was to measure basal tryptase levels in patients with Hymenoptera venom allergy and investigate the possible correlation between these levels and the severity of sting reaction.
Methods: One hundred nine patients were included in the study. Sixty-three were wasp venom-allergic and 46 were honey bee venom-allergic. Basal serum tryptase levels were measured by UniCAP.
Results: Basal serum tryptase levels were elevated in 12 (11%) of the 109 patients. Levels were 5.14 µg/L (3.62-5.84), 5.3 µg/L (2.94-6.54), 5.18 µg/L (3.71-6.25), and 6.98 µg/L (4.78-12.6), for patients with sting reactions of grade I, II, III and IV (as classified by Mueller), respectively. Basal serum tryptase levels correlated significantly with the sting reaction severity ($r = 0.2752$; $P = .004$) and with age ($r = 0.2906$; $P = .002$). Sting reaction severity also correlated with age ($r = 0.3654$; $P = .001$).
Conclusions: Basal serum tryptase levels were found to be elevated in 11% of venom allergic patients and correlated significantly with both sting reaction severity and age.

Key words: Tryptase. Hymenoptera venom allergy.

Resumen

Antecedentes: La gravedad de la reacción tras una picadura de himenópteros se ha relacionado con la presencia de niveles elevados de triptasa sérica. El propósito del estudio fue cuantificar los niveles basales de triptasa en pacientes con alergia al veneno de himenópteros e investigar la posible correlación entre estos niveles y la gravedad de la reacción a la picadura.
Métodos: Participaron en el estudio 109 pacientes. De éstos, 63 eran alérgicos al veneno de avispa y 46 al de la abeja de la miel. Los niveles basales de triptasa sérica se cuantificaron mediante UniCAP.
Resultados: Los niveles basales de triptasa sérica fueron elevados en 12 (11%) de los 109 pacientes. Los niveles fueron de 5,14 µg/L (3,62-5,84), 5,3 µg/L (2,94-6,54), 5,18 µg/L (3,71-6,25) y 6,98 µg/L (4,78-12,6) en los pacientes con reacciones a las picaduras de grado I, II, III y IV (según la clasificación de Mueller), respectivamente. Los niveles basales de triptasa sérica se relacionaron significativamente con la gravedad de la reacción a la picadura ($r = 0,2752$; $P = 0,004$) y con la edad ($r = 0,2906$; $P = 0,002$). La gravedad de la reacción a la picadura también se relacionó con la edad ($r = 0,3654$; $P = 0,001$).
Conclusiones: Los niveles basales de triptasa sérica fueron elevados en un 11% de los pacientes alérgicos al veneno y se correlacionaron significativamente tanto con la gravedad de la reacción a la picadura como con la edad.

Palabras clave: Triptasa. Alergia al veneno de himenópteros.

Introduction

Tryptase is a predominant protease of human mast cells and presents in three isophorms: α-tryptase, pro-ß-tryptase and ß-tryptase. α- and pro-ß-tryptase are enzymatically inactive and released continuously. A persistent rise in serum α-tryptase is an indicator of an elevated number of mast cells, and thus may indicate mastocytosis [1]. ß-tryptase is stored in mast cell granula and is released under conditions of extensive mast cell degranulation [2].

Tryptase is an indicator of mast cell involvement in a variety of clinical conditions. It has been reported to be increased in bronchoalveolar lavage during allergen challenge and in nasal fluid during allergic rhinitis [3, 4]. Elevated levels of tryptase have also been observed in patients with severe anaphylactic reactions [5], less often after food intake [6]. Furthermore, a primary involvement of increased tryptase levels in Hymenoptera allergic individuals has recently been hypothesised [7, 8].

Hymenoptera venom allergy is a life threatening allergy. Clinical symptoms comprise four classes according to Mueller [9]. Systemic anaphylactic reactions constitute 0.3%-7.5% of allergic reactions to Hymenoptera venoms [10]. The efforts of clinicians have focused on searching for a definitive diagnostic test which could accurately predict which group of patients is at high risk for severe reactions.

The aim of the present study was to determine basal tryptase levels in patients with Hymenoptera venom allergy and investigate the possible correlation between these levels and the severity of sting reaction.

Materials and Methods

Patients

One hundred and nine venom-allergic patients were consecutively selected for the study based on a history of reaction after a sting (grades of severity: I, II, III, IV according to Mueller [9]), positive skin intracutaneous tests and/or venom specific serum immunoglobulin (Ig) E (UniCap System, Pharmacia, Uppsala, Sweden).

The study was approved by the local ethics committee (CR-I-003/156/2005). All participants provided written informed consent.

Skin Tests

All patients underwent intracutaneous skin tests using 10-fold dilutions of wasp and honey bee extracts (Venomenhal, Haarlem, Netherlands) with concentrations ranging from 10^{-6} to 10^{-3} g/L as described previously [11]. Results were considered with a weal of 5 mm or more in diameter with erythema after 15 minutes at a concentration of 10^{-3} g/L or less.

Blood Samples

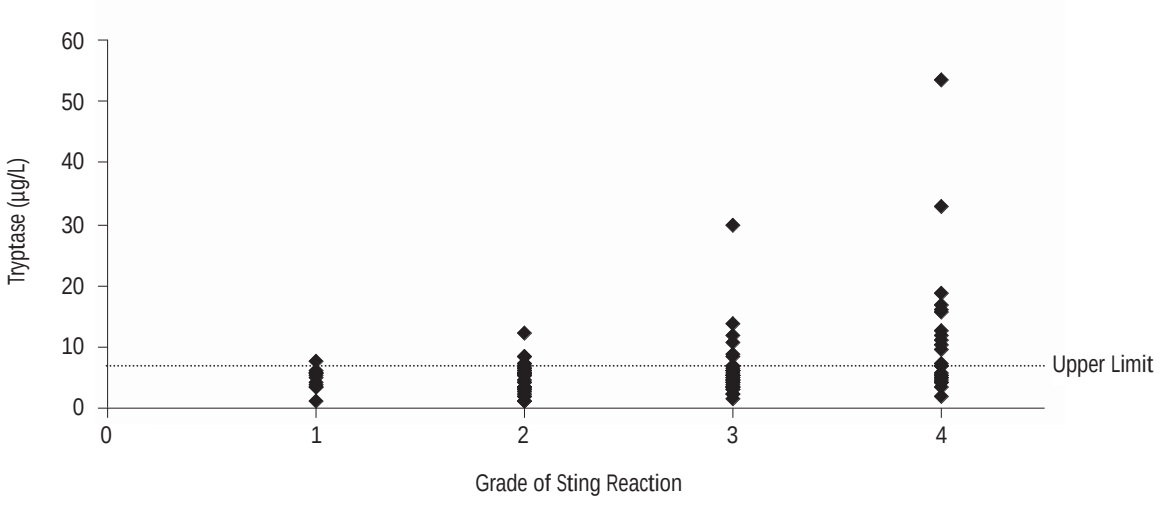
Blood samples were collected between 7 and 9 am, at least four weeks after the Hymenoptera sting. Immediately after centrifugation at 1500*g* for 10 minutes, the sera were aliquoted and frozen at –80 °C until testing.

Basal Serum Tryptase Levels

Basal serum tryptase levels were measured using the UniCAP Tryptase Fluoroenzymeimmunoassay (Pharmacia, Uppsala, Sweden). The detection limit was <1.0 µg/L. Levels of 11.4 µg/L and more were considered to be elevated.

Statistical Analysis

Data are presented as median and 25th-75th percentiles. The data were not normally distributed and nonparametric statistics were employed. Mann-Whitney U Wilcoxon Rank Sum W Test



Basal serum tryptase levels and sting reaction severity.

Table 1. Patient Characteristics (n=109)*

	Wasp-Allergic Subjects	Honey Bee-Allergic Subjects
Patients, n (%)	63/109 (57.8)	46/109 (42.2)
Age, y	36.03 ± 13.3	37.2 ± 11.8
Mean age of patients reacting with grade, y		
I	24.88 ± 4.5	23.75 ± 4.7
II	33.86 ± 10.7	37.3 ± 11.6
III	37.62 ± 13.8	37.4 ± 12.0
IV	44.67 ± 15.0	40.92 ± 11.4
Sex (female/male)	42/21	22/24
sIgE (kU/L)	1.97 (0.35-100)	3.41 (0.35-85.4)
Tryptase level, µg/L	5.46 (1-53.5)	5.24 (1-33)
Patients with increased tryptase levels, n (%)	9/63 (14.3%)	3/46 (6.5%)
Patients reacting with grade, n (%)		
I (12) (11%)	8 (12.7%)	4 (8.7%)
II (35) (32.1%)	22 (34.9%)	13 (28.3%)
III (37) (33.9%)	21 (33.3%)	16 (34.8%)
IV (25) (22.9%)	12 (19%)	13 (28.3%)

* sIgE indicates specific immunoglobulin E.
Values one mean (±) unless otherwise stated.

(SPSS Software) was used to compare patients with regard to basal tryptase level and sting severity. Spearman's non-parametric rank correlation test was used to evaluate the correlation between basal serum tryptase levels, the severity of reaction after sting, age and sIgE. Multiple stepwise regression analysis was used to further check the correlation between sting reaction severity, tryptase levels and age. χ^2 test was used to compare patients with regard to sex, age, tryptase level, severity of reaction after sting. *P* values less than .05 were considered significant.

Results

Patients

Patient characteristics are shown in Table 1. Of the 109 patients 63 (57.8%) were wasp-allergic and 46 (42.2%) were honey bee-allergic. Wasp-and honey bee-allergic subjects did not differ with regard to age and tryptase levels. In the wasp-allergic group there were significantly more women than men (42 vs 21; $P = .038$).

Table 2. Description of Patients With Increased Basal Serum Tryptase

Tryptase Level, µg/L	Grade of Reaction	Culprit Insect
12.6	IV	wasp
29.9	III	wasp
18.9	IV	wasp
13.7	III	wasp
11.9	III	wasp
12.4	II	wasp
33	IV	honey bee
15.6	IV	honey bee
11.9	IV	honey bee
16.1	IV	wasp
16.7	IV	wasp
53.5	IV	wasp

Basal Serum Tryptase Levels

The description of patients with elevated basal serum tryptase is given in Table 2. Basal serum tryptase levels were elevated in 12 (11%) of the 109 subjects. From this group of 12 patients with increased basal tryptase levels, 7 had a severe reaction (grade IV) after a sting, 4 a moderate reaction (grade III), and 1 a mild reaction (grade II). Elevated serum tryptase was observed in 9 wasp and 3 honey bee-allergic subjects. In 3 of the 109 patients basal tryptase levels were border line (10.6 µg/L; 11.1 µg/L; 10.3 µg/L). However, all these patients had a severe reaction after a sting (grades III, IV and IV, respectively).

Basal serum tryptase levels were 5.14 µg/L (3.62-5.84), 5.3 µg/L (2.94-6.54), 5.18 µg/L (3.71-6.25), 6.98 µg/L (4.78-12.6), for patients with sting reactions of grades I, II, III and IV, respectively. The median tryptase level in patients with grade IV reactions was higher than those reacted with grade I, II and III reactions ($P = .027$, $P = .004$, $P = .013$ for grade I vs grade IV, grade II vs grade IV, grade III vs grade IV, respectively).

Basal serum tryptase levels correlated significantly with the sting reaction severity (r=0.2752; P= .004) (Figure 1) and with age (r=0.2906; P= .002). Sting reaction severity also correlated with age (r=0.3654; P= .001). To further check the correlation between sting reaction severities, tryptase levels and age, multiple stepwise regression analysis was performed. When sting reaction (grades I, II, III, IV) was adjusted for age, r=0.38 with a shared variance of 14.6 %. When we adjusted sting reaction for both age and tryptase level, r=0.46 with a shared variance of 21.1 %.

In patients with honey bee allergy, but not in wasp-allergic subjects, sIgE correlated with sting reaction severity (r=0.2417; P=.012). Sex did not affect basal serum tryptase levels or reaction severity.

Discussion

The diagnosis of venom allergy is based on a history of systemic reaction after a sting, positive intracutaneous skin

tests and/or serum specific IgE [10]. Recently new methods for the diagnosis of venom allergy have been proposed. Immunoblotting, basophil activation tests and tryptase represent the most promising diagnostic tools [8,12-15]. Identifying those people with increased risk of severe reaction after a sting is of great clinical importance. Tryptase is a marker of mast cell degranulation and it has been suggested that tryptase levels should be determined in patients with severe reactions after a sting [10].

In the present paper we have demonstrated increased basal serum tryptase levels in 11% (12/109) of patients allergic to wasp or honey bee venoms. Similarly, Haeberli et al [8] demonstrated increased tryptase in 7.3% of venom-allergic patients. In our study basal serum tryptase levels correlated significantly with sting reaction severity. Thus, we have confirmed and extended previous results pointing to the role of tryptase in the severity of sting reactions. Furthermore, we found that tryptase and sting reaction severity increased in relation to age. It has been suggested that older people are at greatest risk of a severe reaction after a sting [16-18], and even than older age could be an independent predictor of reaction severity [19]. Therefore, in patients of older age, as is the case with subjects with elevated tryptase levels, a long-term or lifelong treatment should be considered [20].

Elevated basal serum tryptase levels were found in 14.3% (9/63) of wasp-allergic patients and in 6.5% (3/46) of bee-allergic subjects. Thus, it can be seen that in the group with increased tryptase levels 75% had a wasp allergy. This is consistent with the previous observation also showing significantly more wasp-than honey bee-allergic individuals with increased tryptase levels [8]. However, due to the small number of subject this observation should be further clarified.

Based on a clinical history it is not obvious if only patients with tryptase levels greater than 11.4 µg/L are at a higher risk of a severe reaction after a sting. Recently, Sturm et al [21] have demonstrated that also patients with level > 6.6 µg/L have a comparably high risk for severe sting reactions. Moreover, it has been suggested that note should be taken when tryptase levels are between 8 and 11.4 µg/L [22]. In our study, in the group of subjects with tryptase levels of less than 11.4 µg/L, 34 (31.2%) had a grade III reaction and 17 (15.6%) had a grade IV reaction. Therefore, a decrease in the upper limit of tryptase levels should be further discussed.

Allergen specific immunotherapy constitutes the only possible method of successful treatment of allergic patients. It restores the impaired immunity by providing allergen tolerance. The exact mechanism of successful immunotherapy is still unknown. Recently, the role of antigen specific T regulatory cells has attracted much interest. The production of IL-10 and/or TGF-ß, the influence on effector cells of allergic inflammation, that is, mast cells, basophils and eosinophils, the suppression of IgE synthesis and induction in IgG4 and IgA production, seem to play a major role in the mechanisms responsible for the positive effect of immunotherapy [23]. It is conceivable that venom immunotherapy also causes changes in tryptase levels. However, this possibility has been excluded as it has been shown that venom immunotherapy does not influence serum tryptase levels [8].

One possible limitation of our study is the lack of histological examination in patients with increased basal serum tryptase levels. However, none of these patients showed any clinical symptoms of mastocytosis, such as the typical cutaneous lesions of urticaria pigmentosa, telangiectasia macularis eruptive perstans or gastrointestinal, pulmonary or skeletal system disorders [24]. Mastocytosis is a very rare disease (1 in 1000-8000 patients) [7], and the exact identification of the disease is often difficult and it may be misdiagnosed [25]. As in most patients with cutaneous mastocytosis without systemic involvement, serum α- and ß-tryptase do not exceed normal ranges [24], and normal serum levels do not exclude mastocytosis [26], in our patients with increased tryptase levels further examinations should be undertaken, especially in regard to proposed venom immunotherapy.

In conclusion, basal serum tryptase levels were elevated in 11% of venom allergic patients and this correlated significantly with sting reaction severity and age. Thus, from current studies and also previous reports, a complex but consistent picture emerges for the role of tryptase in venom allergic patients. A decrease in the upper limit of tryptase levels should also be further discussed.

References

1. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, Metcalfe DD. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest. 1995;96:2702-10.
2. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest. 1989;83:1551-5.
3. Wenzel SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am Rev Respir Dis. 1988;137:1002-8.
4. Rasp G, Hochstrasser K. Tryptase in nasal fluid is a useful marker of allergic rhinitis. Allergy. 1993;48:72-4.
5. Brown SG. Cardiovascular aspects of anaphylaxis: implications for treatment and diagnosis. Curr Opin Allergy Clin Immunol. 2005;5:359-64.
6. Golden DB. Patterns of anaphylaxis: acute and late phase features of allergic reactions. Novartis Found Symp. 2004;257:101-10.
7. Ludolph-Hauser D, Rueff F, Fries C, Schopf P, Przybilla B. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet. 2001;357:361-2.
8. Haeberli G, Bronnimann M, Hunziker T, Muller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy. 2003;33:1216-20.
9. Mueller HL. Diagnosis and treatment of insect sensivity. J Astma Res. 1966;3:331-3.
10. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy. 2005;60:1339-49.

11. Thurnheer U, Muller U, Stoller R, Lanner A, Hoigne R. Venom immunotherapy in Hymenoptera sting allergy. Allergy. 1983;38:465-75.
12. Pereira Santos MC, Pedro E, Spinola Santos A, Branco Ferreira M, Palma Carlos ML, Palma Carlos AG. Immunoblot studies in allergic patients to hymenoptera venom before and during immunotherapy. Allerg Immunol. 2005;37:273-8.
13. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? Clin Exp Allergy. 2004;34:332-9.
14. Sturm GJ, Bohm E, Trummer M, Weiglhofer I., Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. Allergy. 2004;59:1110-17.
15. Binder M, Fierlbeck G, King T, Valent P, Buhring HJ. Individual hymenoptera venom compounds induce upregulation of the basophil activation marker ectonucleotide pyrophosphatase/ phosphodiesterase 3 (CD203c) in sensitized patients. Int Arch Allergy Immunol. 2002;29:160-8.
16. Lockey RF, Turkeltaub PC, Baird-Warren IA, Olive CA, Olive ES, Peppe BC, Bukantz SC. The Hymenoptera venom study I, 1979-1982: demographics and history-sting data. J Allergy Clin Immunol. 1988;82:370-81.
17. Lantner R, Reisman RE. Clinical and immunologic features and subsequent course of patients with severe insect-sting anaphylaxis. J Allergy Clin Immunol. 1989;84:900-6.
18. Przybilla B, Ring J, Grieshammer B. Association of features of atopy and diagnostic parameters in hymenoptera venom allergy Allergy. 1991;46:570-6.
19. Brown SG. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol. 2004;114:371-6.
20. Bonifazi, F, Jutel M, Bilo BM, Birnbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;60:1459-70.
21. Sturm G, Wiednig M, Strele A, Schuster C, Aberrer W. Baseline serum tryptase levels and the severity of sting reactions in Hymenoptera venom allergy. XXV Congress of EAACI, Vienna 2006, Abstract book page 48.
22. Haeberli G, Meier R, Helbling A, Muller U. Severity of Hymenoptera venom allergy in correlation to basal tryptase levels. XXV Congress of EAACI, Vienna 2006, Abstract book page 48.
23. Akdis CA, Blaser K, Akdis M. Mechanisms of allergen-specific immunotherapy. Chem Immunol Allergy. 2006;91:195-203.
24. Ispas L, Henriksen RA, Metzger WJ. The many faces of systemic mastocytosis. Ann Allergy Asthma Immunol. 2001;87:6-14.
25. Biedermann T, Rueff F, Sander CA, Przybilla B. Mastocytosis associated with severe wasp sting anaphylaxis detected by elevated serum mast cell tryptase levels. Br J Dermatol. 1999;141:1110-12.
26. Koide T, Nakajima T, Makifuchi T, Fukuhara N. Systemic mastocytosis and recurrent anaphylactic shock. Lancet. 2002;359:2084.

Manuscript received August 28, 2006; accepted for publication October 24, 2006.

Iwona Kucharewicz

Department of Allergology and Internal Medicine
Medical University of Bialystok
Sklodowska-Curie Street 24a
15-276 Bialystok, Poland
E-mail: ikuchar@poczta.onet.pl