ORIGINAL ARTICLE

Serum Tryptase Concentrations in Beekeepers With and Without Hymenoptera Venom Allergy

F Carballada,[1] M Alonso,[2] L Vizcaino,[2] V Coutinho,[2]
R Núñez,[1] C Vidal,[3] M Boquete,[1] A González-Quintela[2]

[1]Department of Allergy, Hospital Lucus Augusti, Lugo, Spain
[2]Department of Medicine, Hospital Clínico Universitario, Santiago de Compostela, Spain
[3]Department of Allergy, Hospital Clínico Universitario, Santiago de Compostela, Spain

Abstract

Background: Increased tryptase concentrations are a risk marker for the severity of reactions to Hymenoptera stings or venom immunotherapy.
Objective: To investigate serum tryptase concentrations in beekeepers with and without Hymenoptera venom allergy (HVA).
Methods: Serum tryptase concentrations were measured in adult patients with HVA (n=91, 37 of whom were beekeepers), beekeepers without HVA (n=152), and control individuals from the general adult population (n=246).
Results: Multivariate analyses revealed that serum tryptase levels were positively associated with beekeeping activities ($P<.001$) and HVA ($P<.001$). Tryptase levels were also positively associated with age ($P<.001$) and male sex ($P=.02$), and negatively associated with alcohol consumption ($P=.002$).
Conclusions: Beekeeping and HVA are independently associated with increased concentrations of serum tryptase.

Key words: Tryptase. Hymenoptera. Beekeeper. Venom allergy.

Resumen

Antecedentes: Los niveles séricos elevados de triptasa son un marcador de gravedad de las reacciones a las picaduras de himenópteros y de las reacciones a la inmunoterapia específica.
Objetivo: Investigar los niveles séricos de triptasa en apicultores alérgicos y no alérgicos al veneno de himenópteros.
Métodos: Se determinó la triptasa sérica en pacientes adultos con alergia al veneno de himenópteros (n=91, 37 de los cuales eran apicultores), apicultores sin alergia al veneno de himenópteros (n=152), y en controles de una población general adulta (n=246).
Resultados: En los análisis multivariante, se observó que las concentraciones de triptasa sérica estaban positivamente asociadas con el hecho de ser apicultor ($P<.001$) y con el hecho de ser alérgico al veneno de himenópteros ($P<.001$). Los niveles de triptasa sérica tambien se asociaron positivamente con la edad ($P<.001$) y el sexo masculino ($P=.02$), y negativamente con el consumo de alcohol ($P=.002$).
Conclusiones: La apicultura y la alergia al veneno de himenópteros se asocian independientemente con concentraciones elevadas de triptasa sérica.

Palabras clave: Triptasa. Himenópteros. Apicultura. Alergia a venenos.

Introduction

Serum or plasma concentrations of total tryptase are routinely used in clinical practice as a marker of mast cell burden and/or activation [1,2]. Tryptase concentrations are thus useful for the diagnosis and follow-up of clonal mast cell diseases (systemic mastocytosis and related disorders) [1,3], additional myeloid neoplasms [4], and mast cell activation syndrome [5]. Concentrations transiently increase following mast cell degranulation and are therefore useful in the diagnosis of systemic anaphylaxis [1,2]. In patients with Hymenoptera venom allergy (HVA), increased baseline tryptase concentrations indicate a risk of severe reactions, even during immunotherapy [6-8], and they may also be a marker

of an underlying clonal mast cell disorder [9]. The relationship between mast cells and HVA is therefore intriguing. The purpose of the present study was to investigate serum tryptase concentrations in beekeepers, who are frequently exposed to Hymenoptera stings. We compared serum tryptase concentrations in beekeepers with and without HVA, and further compared their results with those of adults in the general population.

Material and Methods

Study Populations

This study took advantage of 3 previous surveys that were primarily conducted to investigate factors associated with immunoglobulin (Ig) E–mediated carbohydrate sensitization [10-12]. The study profiles and main demographic characteristics are presented in Figure 1. The first survey was performed in beekeepers from a professional association in the area of Lugo, Spain [10]. All of the individuals answered a structured questionnaire and underwent laboratory determinations as reported elsewhere [10]. Reactions to the beekeepers' last bee sting were classified as none, small local, large local (diameter >10 cm and peaking at 24-48 hours), or systemic [10]. From the initial sample (n=158), 3 individuals were excluded because they had a prior diagnosis of HVA and were currently undergoing immunotherapy. In addition, systemic reactions were newly diagnosed in 3 beekeepers, who were therefore included in the group of patients with HVA (see below).

The second survey was performed in patients with HVA from a specialized clinic [11]. Individuals were seen for the first time in the clinic and diagnosed as having HVA following standard procedures [11]. The 3 beekeepers with HVA from the first study were added to this group, which thus comprised 91 individuals (Figure 1). Of these, the vast majority (n=69, 75.8%) were allergic to honeybee venom, 18 (19.8%) were allergic to yellow jacket venom, and the remaining 4 were allergic to both venoms. The severity of the anaphylactic reaction was classified according to Mueller grades [13]. None of the patients in this group reported a recent (<15 days) Hymenoptera sting, and 37 reported a history of beekeeping activities.

The third study corresponded to a control sample of adult individuals from a neighboring area who were not allergic to Hymenoptera venom and had not performed beekeeping

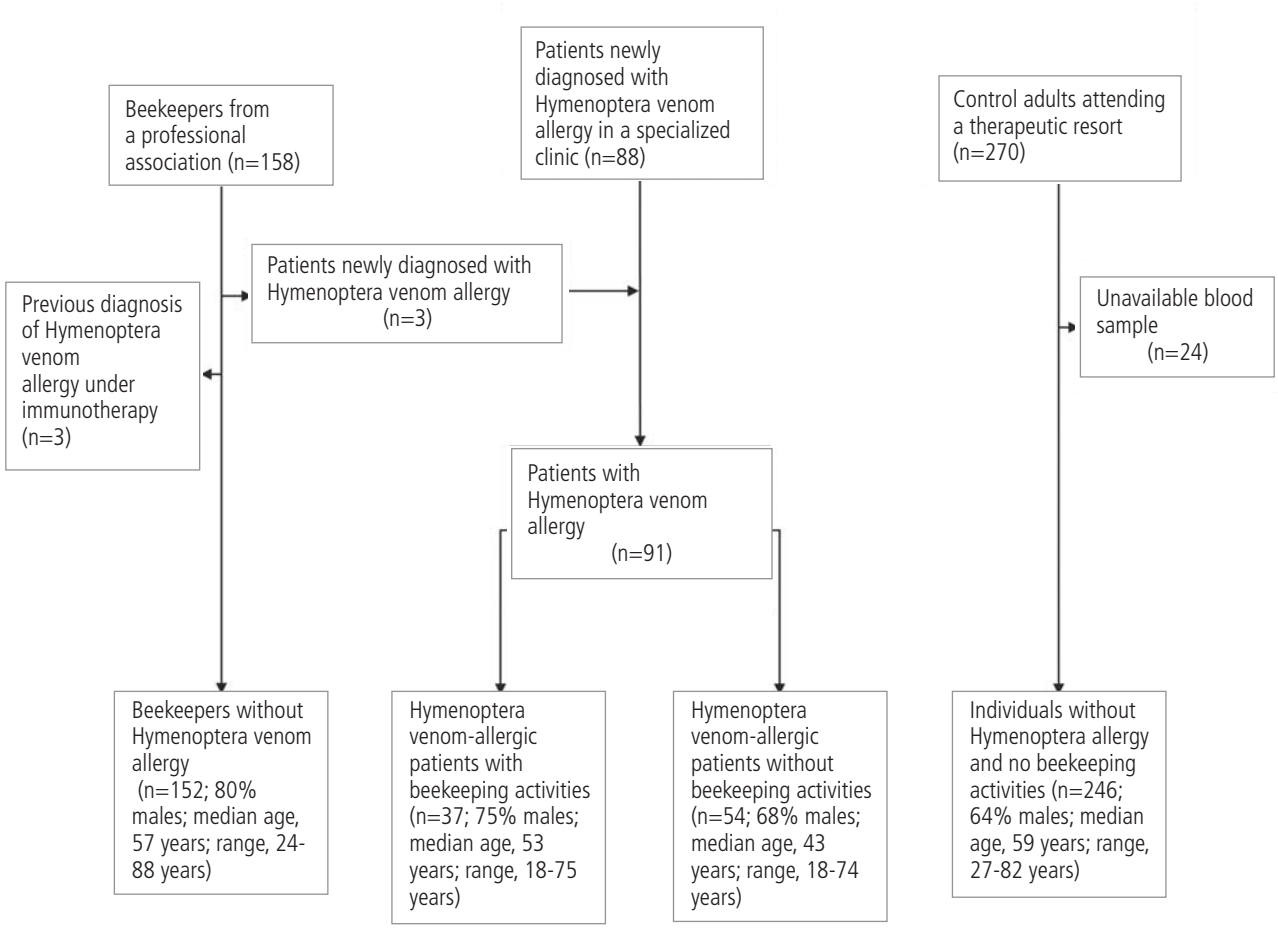

Figure 1. Study profile.

activities [12]. They were studied by means of a structured questionnaire and laboratory determinations while attending a therapeutic spa resort, as reported elsewhere [12]. Serum samples for tryptase determination were available for 246 of these 270 control individuals (Figure 1).

All of the individuals consented to participate in the study, which conformed to the principles of the Helsinki declaration and was approved by the Santiago de Compostela institutional review board.

Serum Tryptase Determination

Serum tryptase was assayed with ImmunoCAP Tryptase (Phadia, now Thermo Fischer Scientific) which measures total tryptase; i.e., all pro-forms and mature forms of ß- and α-tryptase [1,14]. The reportable range is 1 to 200 µg/L. The manufacturer recommends an upper reference level of 10 µg/L, based on the results from a sample of 126 healthy individuals [14]. Some authors have used the 95th percentile in that sample (11.4 µg/L) as the upper reference level [9], while others have adopted the 95% upper confidence limit for the mean (13.5 µg/L) [6]. Yet other authors have suggested 15 µg/L as the upper reference level [1].Tryptase levels of above 20 µg/L are considered a diagnostic criterion for systemic mastocytosis [3].

Covariates

Results were adjusted for demographic variables (age and sex) and lifestyle factors (alcohol consumption and smoking). Consumers of at least 1 cigarette per day were considered smokers. Alcohol consumption was evaluated as the number of standard drinking units (glasses of wine [~10 g], bottles of beer [~10g], and spirits [~10g]) consumed regularly per week. Participants were considered atopic when they had positive skin prick tests to a panel of common aeroallergens or positive serum specific IgE in a multiallergen test (Phadiatop, Phadia), as reported elsewhere [10-12]. No cases of food allergy or systemic mastocytosis were recorded in the study participants.

Statistical Analyses

The Mann-Whitney U test was used to compare tryptase concentrations between groups, the χ^2 test to compare proportions, and the Spearman rank test to assess correlation. Linear regression was used for multivariate analyses; for this purpose, tryptase concentrations (dependent variable) were $\log_{10}$-transformed in order to normalize their distributions. Cases with undetectable tryptase concentrations (n=14) were assigned an arbitrary value of 1 µg/L. All covariates were forced to enter the equation.

Results

Beekeeping and HVA were independently associated with increased serum tryptase concentrations (Figure 2). Among individuals without HVA, tryptase levels were higher in beekeepers than in the general population. Among those with HVA, tryptase levels were higher in those who performed beekeeping activities than in those who did not. Individuals with HVA had higher serum tryptase concentrations than individuals without, regardless of whether or not they undertook beekeeping activities (Figure 2). The prevalence of abnormally high (>10 #mg/L) tryptase concentrations was similar in patients with HVA (5.5%), beekeepers without HVA (3.9%), and control individuals (4.1%, *P*=.82).

Multivariate analyses revealed that serum tryptase levels were positively associated with beekeeping, HVA, and age, and inversely associated with alcohol consumption (Table 1). Levels tended to be positively associated with smoking and were not significantly associated with atopy (Table 1).

Among those with HVA, tryptase levels were not significantly associated with the severity of systemic reactions to stings, either in the group as a whole or after adjusting for a history of beekeeping (Figure 3). Beekeeping tended to be associated with higher serum tryptase concentrations, independently of the severity of the systemic reaction (Figure

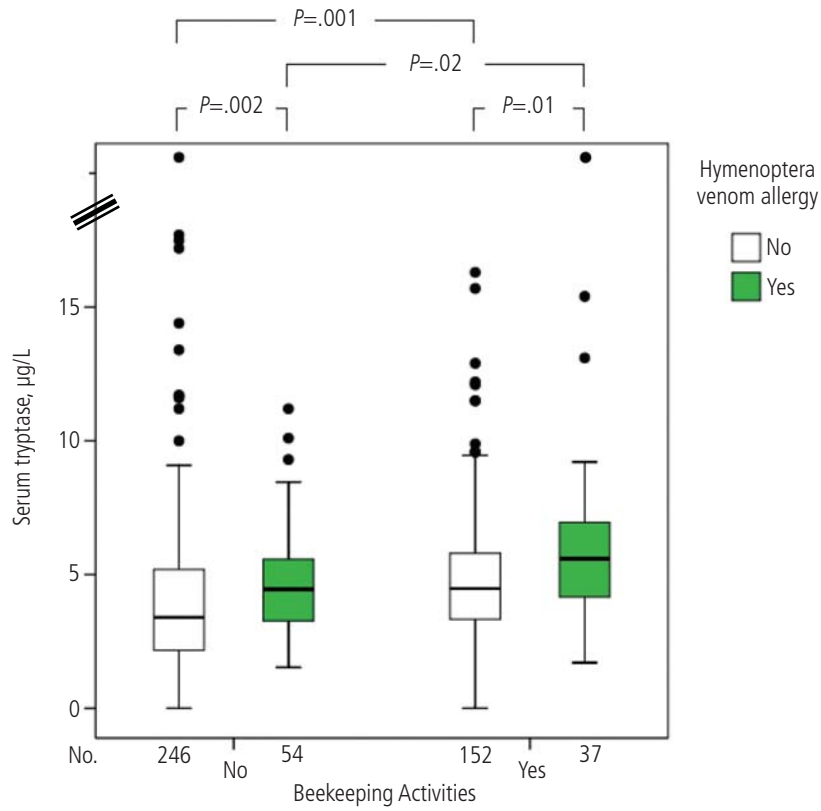

Figure 2. Serum tryptase concentrations among participants stratified by beekeeping activities and Hymenoptera venom allergy.

Table 1. Multivariate Analysis (Linear Regression) of Factors Associated With Serum Tryptase Concentrations[a]

Covariates	Coefficient (Slope)	Standard Error	*P* Value
Age, y	0.004	0.001	<.001
Sex			
Female	(reference)		
Male	0.070	0.030	.02
Alcohol consumption, units/wk	-0.002	0.001	.002
Current smoking			
No	(reference)		
Yes	0.068	0.039	.08
Beekeeping activities			
No	(reference)		
Yes	0.104	0.025	<.001
Hymenoptera venom allergy			
No	(reference)		
Yes	0.138	0.033	<.001
Atopy			
No	(reference)		
Yes	-0.005	0.031	.87
Constant (intercept)	0.295	0.061	

[a] Tryptase concentrations (dependent variable) were $\log_{10}$-transformed in order to normalize their distribution. For this purpose, cases with undetectable tryptase concentrations were assigned an arbitrary value of 1 µg/L. All covariates entered the equation. Age was introduced in years and alcohol consumption was introduced in units/week. The remaining variables were introduced as "1= present or yes" and "0=absent or no". The model explained 11% of the variability of serum tryptase concentrations (R square, 0.11).

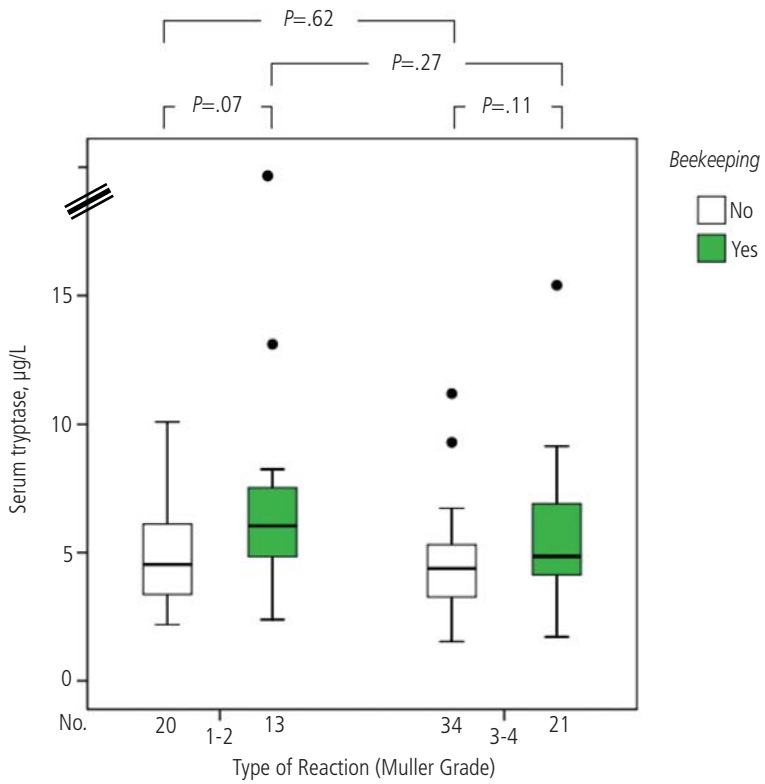

Figure 3. Serum tryptase concentrations among Hymenoptera *venom*-allergic individuals stratified by the severity of systemic reaction and beekeeping activities. Grades 1-2 and 3-4 were grouped due to the small number of individuals in each group.

Table 2. Correlation Between Serum Tryptase Concentrations and Epidemiological and Immunological Factors Related to Beekeeping[a]

	Duration of Beekeeping Activities, y	No. of Beehives	No. of Bee Stings in the Last Year	Time Elapsed Since the Last Sting, mo	IgE to HBV[b] kU_A/L	IgG to HBV mg_A/L	IgG4, to HBV mg_A/L
Median (range)	20 (2-75)	9 (1-1000)	4 (0-300)	6 (<1-46)	0.55 (0.03->100)	11.6 (<2-135)	3.22 (0.02-30)
Correlation coefficient with serum tryptase	0.165	-0.011	-0.014	-0.087	-0.093	0.115	0.19
P value	.04	.89	.86	.28	.25	.15	.01

Abbreviations: HBV, honeybee venom; Ig, immunoglobulin.
[a]Data available for 152 beekeepers without Hymenoptera venom allergy.
[b]HBV antibodies were detected in the UniCAP 250 system (Phadia), as reported elsewhere [10].

3). Serum tryptase concentrations tended to be higher in patients allergic to honeybee venom than in patients allergic to yellow jacket venom (median, 4.85 μg/L and range, 1.71-26.3 μg/L, vs median, 4.45 μg/L and range, 1.60-6.39 μg/L; *P*=.08).

Among beekeepers without HVA, tryptase levels were not associated with the number of beehives, average number of bee stings per year, or time elapsed since the last bee sting (Table 2). We observed that levels tended to be associated with the duration of beekeeping activities, but this association was greatly attenuated after adjusting for age (data not shown). In the same group (beekeepers without HVA), serum tryptase levels were similar among individuals with no reaction, a small local reaction, or a large local reaction to the last honeybee sting (Figure 4).

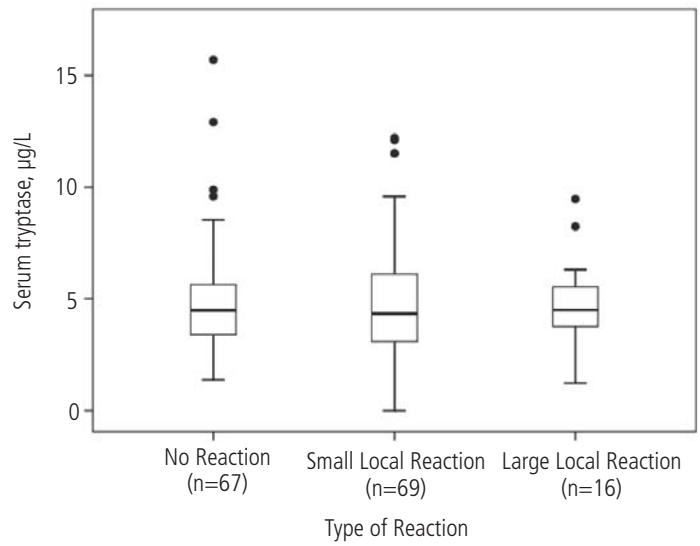

Figure 4. Serum tryptase concentrations among beekeepers without Hymenoptera venom anaphylaxis stratified according to the local skin reaction to the last honeybee sting. The differences between groups were not statistically significant (*P*>.5).

Discussion

The present study shows that beekeeping and HVA are positively and independently associated with serum tryptase concentrations. These associations were independent of potential confounders. In the general population, serum tryptase concentrations increase with age [15,16]. A similar age-related increase was observed in patients with HVA in a previous study [17] and in the present study. In the general population, there are no clear sex-related differences in serum tryptase levels, which tend to be higher in males than in females [15,16]. This was also the case in our study. Likewise, serum tryptase concentrations are similar in atopic and nonatopic individuals [15,16]. Serum tryptase concentrations increase in relation to body mass and are higher in overweight or obese individuals than in lean individuals [15,16]. Similarly to previous studies [15,16], atopy (positive skin prick tests or positive serum IgE specific to aeroallergens) was not significantly associated with serum tryptase levels. In 2 previous studies, alcohol consumption was associated with low serum tryptase levels [15,16], which is consistent with the results of our study.

From a clinical standpoint, the differences in serum tryptase concentrations among groups defined by beekeeping and HVA are small. In fact, the factors studied explained little of the variation in tryptase concentrations. Furthermore, the prevalence of abnormally increased tryptase concentrations was similar between patients with and without HVA and also between beekeepers and nonbeekeepers. However, the association beween tryptase levels and HVA and beekeeping may be interesting from a mechanistic standpoint. The increase in serum tryptase concentrations among patients with HVA is consistent with previous reports of a risk of severe reactions to stings or venom immunotherapy in individuals with high tryptase levels [6-9]. It is noteworthy that this risk is evident even in patients with normal baseline tryptase concentrations [18].

To the best of our knowledge, an association between beekeeping and increased tryptase concentrations has not been previously reported, and may add information to what is known about the complex relationship between Hymenoptera

venom exposure and mast cells [19]. The reasons for such an association are not known. The association observed in our study was independent of known confounders, but there could be unknown confounders associated with beekeeping that induced the increased tryptase concentrations observed in this population. The additional limitations of observational, cross-sectional studies should be taken into account. Alternatively, increased tryptase levels in beekeepers could have a biological explanation. Mast cell products neutralize major toxins in bee venom and protect against venom toxicity in animal models [20,21], and in addition, repeated venom exposure through natural bee stings induces significant immune modulation [22]. Hypothetically, a reactive increase in mast cell burden after repeated exposure could explain part of the increased risk for HVA in beekeepers [23-26]. However, these are speculations and the exact mechanism underlying high tryptase concentrations in beekeepers should be explored in further studies.

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Arturo González-Quintela
Servicio de Medicina Interna
Hospital Clinico Universitario
15706 Santiago de Compostela, Spain
E-mail: arturo.gonzalez.quintela@usc.es

