

Serum Concentrations of Tryptase in Children

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Background: Mast cells are dispersed in many tissues, especially in digestive and respiratory-tract mucosal membranes. Tryptase, considered a marker of mast-cell activity, is the most important protease released from these cells during degranulation. Tryptase concentration is mainly accessed in anaphylaxis and mastocytosis, being one diagnostic criteria of this disease. There are no data concerning tryptase activity in healthy children in the current literature.

Aim: The aims of this study were the analysis of concentrations of serum tryptase in healthy children, and determining reference values of the enzyme at different developmental ages.

Materials and methods: The investigated group consisted of 131 healthy children (75 girls, 56 boys) aged 3 months–18 years. The concentration of tryptase in the studied samples was evaluated by the fluoro-immuno-enzymatic method with UniCAP.

Results: The mean concentration of serum tryptase in the studied group was 2.8 ± 2.2 ng/dL; 2.5 ± 2.2 ng/dL in girls and 3.2 ± 2.1 ng/dL in boys.

Conclusion: The upper reference limit of 7.2 ng/dL was lower than in adults.

Introduction

THE CONCENTRATION OF TRYPTASE, an enzyme synthesized by mast cells, is considered a marker of the number and activity of these cells.¹ Mast cells are widely spread throughout tissues, especially at the sites of initial contact with antigen, such as the skin and mucosa of the digestive and respiratory tract. Mast cells derive from multipotent bone-marrow stem cells. During their ontogenesis, they colonize tissues, where they undergo further differentiation and maturation.² These highly specialized cells release an array of biologically active compounds, such as histamine, serotonin, heparin, and cytokines: interleukin (IL)-3, -5, and -6, and tumor necrosis factor (TNF).³ They are stored in mast-cell granules, from which they can be released in the process of degranulation induced by various factors. Tryptase, a neutral protease, also belongs to the group of preformed mediators, secreted by mast cells during the early phase of inflammatory response.⁴ Acting proteolytically, it activates many extracellular peptides, including vasoactive intestinal peptide (VIP) and fibronectin. Furthermore, it is a growth factor for endothelial and epithelial cells, and fibroblasts. It stimulates the release of IL-8 from neutrophils, which results in enhanced migration of inflammatory cells.⁵ Moreover, tryptase stimulates the degranulation of surrounding mast cells, further enhancing the

inflammatory response. The best-known mechanism of mast-cell degranulation is associated with antigen binding to immunoglobulin E anchored at the surface of the cell, which underlies immediate hypersensitivity. Moreover, mast cells are involved in delayed-type hypersensitivity.^{2,6} Involvement in the early, nonspecific phase of inflammatory response seems to be the most important among the roles played by mast cells in the pathogenesis of nonallergic inflammatory conditions. Mast cells show high expression of TLR receptors, which induce their activation upon binding bacterial pathogens.⁷ Furthermore, they can be nonspecifically activated by an array of exogenous and endogenous factors, such as bacteria and their toxins, drugs, substance P, VIP, activated components of the complement (C3a, C4a, C5a), and endorphin.^{2,6} Although tryptase does not represent the most active compound released by mast cells, it constitutes the specific marker of their activation, as it is virtually absent in other cells; basophils are the only cells showing traces of the activity of this enzyme.⁸

Serum concentrations of tryptase and its concentration in other body fluids are analyzed routinely as a marker of anaphylaxis during allergen challenge and finally as a minor criterion of mastocytosis.^{9–12} Mastocytosis is a rare disease caused by hyperplasia and hyperactivation of mast cells diagnosed in both children and adults classified as among myeloproliferative diseases.^{12,13} Moreover, the activity of

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tryptase is determined in other inflammatory conditions, such as inflammatory bowel disease, glomerulonephritis, and rheumatoid arthritis.^{14–16} A role of mast cells and their mediators has also been postulated in the case of noninflammatory conditions, including atherosclerosis and irritable bowel syndrome, and in hematological disorders.^{17–21} However, the results of these studies are inconclusive.

The aim of this study was to analyze the serum activity of tryptase in healthy children and to determine its reference ranges in this group.

Material and Methods

The study included 131 children (75 girls and 56 boys) aged between 3 months and 18 years, previously hospitalized at the Department of Pediatrics, Gastroenterology, Hepatology, and Nutrition of the Medical University of Gdansk between 2009 and 2011. We enrolled only the children in whom the organic background of reported problems was excluded and whose symptoms resolved spontaneously. All the individuals whose symptoms persisted despite the lack of somatic disorders were excluded from the study, as were any patients with dermatological lesions. The reasons for hospitalization of the study participants are summarized in Table 1.

The study group was divided into five age categories (Table 2). After excluding the somatic background of patient condition and normalizing other laboratory parameters, 2 mL of blood was collected from each participant.

Serum concentrations of tryptase were determined using fluoro-immuno-enzymatic assay (FEIA) with a UniCAP Tryptase kit and UniCAP100 analyzer (Phadia, Uppsala, Sweden), following the instructions provided by the manufacturer.

Statistical analysis was conducted with Statistica v10.0 (StatSoft, Inc., Tulsa, OK). Due to the lack of normal distribution of tryptase concentration in the studied group, its values in two and more subgroups were compared with the Mann–Whitney *U*-test and the Kruskal–Wallis test with Tukey and Dunn post-hoc tests. The threshold of statistical significance for all tests was set at $p=0.05$.

The protocol of the study was approved by the Local Bioethical Committee of the Medical University of Gdansk.

Results

Serum concentrations of tryptase documented in various age groups are summarized in Table 3.

TABLE 1. THE CAUSES OF PREVIOUS HOSPITALIZATION IN THE STUDIED GROUP

<i>Cause of hospitalization</i>	<i>Number of patients</i>
Prolonged infant jaundice*	8
Liver damage*	25
Headache*	17
Abdominal pain*	19
Abnormal results of laboratory tests*	23
Other*	20

*After the symptoms have resolved.

TABLE 2. NUMBERS OF PATIENTS IN AGE GROUPS IN BOTH SEXES

	<i>Boys</i>	<i>Girls</i>	<i>Total</i>
< 1 year	6 54.55%	5 45.45%	11
2–3 years	4 30.77%	9 69.23%	13
4–6 years	11 57.89%	8 42.11%	19
7–15 years	22 43.14%	29 56.86%	51
16–18 years	13 35.14%	24 64.86%	37
Total	56	75	131

Statistical analysis did not reveal significant age-related differences in the concentration of the enzyme in the studied group as a whole ($p=0.2209$). However, the median level of tryptase differed significantly between girls from various age categories ($p=0.0089$). Post-hoc analysis revealed that the median concentration of the enzyme in 4- to 6-year-old girls was significantly higher than in those between 16 and 18 years of age ($p=0.0137$). We did not document significant differences between tryptase concentrations of girls from other age categories (Table 4). Furthermore, no significant age-related differences in median concentrations of tryptase were observed in boys ($p=0.0993$; Table 4b). However, the median level of tryptase in boys proved significantly higher than in girls ($p=0.0124$; Table 4).

We used the double standard deviation method to determine the reference values of tryptase concentrations in the studied group. The upper reference limit was 7.2 ng/mL in the whole group: 6.9 ng/mL in girls and 7.4 ng/mL in boys. The reference values in all age subgroups in both genders are presented in Tables 3 and 4.

Discussion

The current study investigates values of tryptase in different age groups from the pediatric population. The role of mast cells is the subject of an increasing number of publications. They deal both with the conditions associated with unquestioned involvement of these cells, such as anaphylaxis and mastocytosis, and with other inflammatory and noninflammatory conditions. This reflects the complex function of mast cells and various mechanisms of their activation. A FEIA with antibody against B12 determinant of tryptase was released by Upjohn Pharmacia in 1994, and is used to date to measure the total concentration of the enzyme. Reference values were also proposed, determined by the manufacturer's study of 126 healthy volunteers aged between 12 and 65 years. The upper normal limit was set to 11.3 ng/mL.²² In the same year, Schwartz et al. published the results of their study of the usefulness of this new method in assessing the risk of anaphylactic reaction. The activity of tryptase in the control group of 56 healthy volunteers participating in this study was 4.9 ± 2.3 ng/mL.²³ In another paper, the same authors proposed a 15 ng/dL upper reference limit of the enzyme concentration.²⁴ However, we lack the data on the reference ranges of tryptase in children and adolescents. This may reflect problems with obtaining

TABLE 3. VALUES OF TRYPTASE IN AGE SUBGROUPS

	<1 year	2–3 years	4–6 years	7–15 years	16–18 years	Whole group
Mean value $\pm$ SD	3.1 $\pm$ 1.3	2.4 $\pm$ 2.4	2.9 $\pm$ 1.7	3.1 $\pm$ 2.3	2.5 $\pm$ 2.3	2.8 $\pm$ 2.2
Range	0.5–5.1	0.7–10.0	0.5–6.6	0.2–9.5	0.1–8.9	0.1–10.0
Median value	3.2	2.3	2.6	2.4	2.0	2.3
95% CI	[2.2, 4.0]	[1.0, 3.9]	[2.1, 3.7]	[2.4, 3.7]	[1.7, 3.3]	[2.4, 3.2]
2 $\times$ SD	2.6	4.8	3.4	4.6	4.6	4.4
Mean value $\pm$ 2 $\times$ SD	5.7	7.2	6.3	7.7	7.1	7.2

SD, standard deviation; CI, confidence interval.

approval for an experiment involving healthy children, especially from the youngest age group. Therefore, most authors who have studied the role of tryptase in various pediatric conditions compared the levels of this enzyme between patients and controls, usually hospitalized at the same institution. However, taking into account the variety and the nonspecific character of mechanisms responsible for mast-cell activation, studies in which the control group was comprised of healthy children should be ascribed a higher value.

In our study, the concentrations of tryptase were determined in children who were previously hospitalized at our clinic for a variety of reasons but who eventually proved to be healthy. Obtaining parental consent for each child's participation in the study and sampling was markedly easier under such circumstances, as the blood concentrations of tryptase were determined in addition to other laboratory parameters, without exposing the patient to additional venipuncture-related stress. Usually, the samples were obtained during follow-up visits, in a day-care unit setting. We excluded children with functional abdominal pain and stress headache, as some neuropeptides associated with these conditions can cause nonspecific mast-cell degranulation. Although our group was relatively large and included 137 patients, some age groups were underrepresented. This explains statistically significant differences observed between median concentrations of tryptase in some age groups of girls, as well as gender-specific differences in this parameter.

The mean concentration of tryptase was 2.8 ± 2.2 ng/mL in the overall studied group, and 2.5 ± 2.2 ng/mL in girls and

3.2 ± 2.1 ng/mL in boys. Upper reference limits were 7.2 ng/mL in the whole group and 6.9 ng/mL and 7.4 ng/mL in girls and boys respectively. Our study revealed that concentrations of tryptase in children are lower than in adults. Consequently, it could be supposed that the level of this enzyme increases with age. However, we did not document such a tendency in our group. Moreover, the levels of tryptase observed in our study were slightly lower than those reported by other authors analyzing the activity of this enzyme in various pediatric conditions.

In the study by Gąsiorowska et al., who analyzed the concentrations of tryptase in children with obstructive bronchitis, the control group comprised 30 patients aged between 1 and 36 months, hospitalized at the same clinic for extrabronchial reasons. The median concentration of tryptase was 3.8 ng/mL, and the analyzed groups did not differ significantly in terms of this parameter.²⁵ In another study, involving patients with mastocytosis, the control group included 30 healthy children aged between 3 months and 17 years; the mean concentration of tryptase in this group was 3.28 ± 3.03 ng/mL.²⁶

Anaphylaxis constitutes the most frequently reported pathology being associated with the release of tryptase from mast cells. The results of many studies, both in adults and in children, confirmed that high baseline concentrations of tryptase constitute the risk factor of severe anaphylactic reaction.²⁷ Pediatric patients who were sensitive to bee venom and experienced severe adverse events during immunotherapy showed significantly higher levels of tryptase than controls. The control group included in the study by

TABLE 4. DISTRIBUTION OF SERUM TRYPTASE CONCENTRATIONS BY AGE SUBGROUPS AND GENDER

	<1 year	2–3 years	4–6 years	7–15 years	16–18 years	Total
<i>Tryptase concentration values in girls</i>						
Mean value $\pm$ SD	2.4 $\pm$ 1.3	2.5 $\pm$ 2.9	3.8 $\pm$ 1.7	2.9 $\pm$ 2.2	1.6 $\pm$ 1.9	2.5 $\pm$ 2.2
Range	0.5–3.9	0.7–10.0	1.9–6.6	0.2–8.5	0.1–8.9	0.1–10.0
Median value	2.5	1.9	3.4	2.4	0.9	2.1
95% CI	[0.8, 4.0]	[0.2, 4.7]	[2.4, 5.3]	[2.1, 3.8]	[0.8, 2.4]	[2.0, 3.0]
2 $\times$ SD	2.6	5.8	3.4	4.4	3.8	4.4
Mean value $\pm$ 2 $\times$ SD	5.0	8.3	7.2	7.3	5.4	6.9
<i>Tryptase concentration values in boys</i>						
Mean value $\pm$ SD	3.7 $\pm$ 1.1	2.3 $\pm$ 1.2	2.2 $\pm$ 1.4	3.2 $\pm$ 2.4	4.1 $\pm$ 2.3	3.2 $\pm$ 2.1
Range	2.3–5.1	1.4–3.8	0.5–4.6	0.7–9.5	0.8–8.1	0.5–9.5
Median value	4.1	2.4	2.1	2.4	3.6	2.7
95% CI	[2.5, 4.9]	[1.3, 3.2]	[1.3, 3.2]	[2.1, 4.3]	[2.7, 5.4]	[2.6, 3.7]
2 $\times$ SD	2.2	2.4	2.8	4.8	4.6	4.2
Mean value $\pm$ 2 $\times$ SD	5.9	4.7	5.0	8.0	8.7	7.4

Yvez et al. comprised 56 healthy children aged between 3 and 17 years; the median concentration of tryptase in this group (2.9 ng/mL) was similar to that documented in our study.²⁸ It is of note that, like in our study, the concentrations of tryptase determined in all the studies mentioned above were lower than in adults. Also, the level of tryptase associated with the risk of anaphylactic reaction in children seems to be lower than in adult patients. The concentrations of tryptase exceeding 7.6 ng/mL, thus only slightly higher than the upper reference limit determined in our study, were revealed to be associated with the risk of severe anaphylactic reaction in children subjected to immunotherapy.²⁹

Mastocytosis is another condition in which the level of tryptase becomes of vital importance. A high level of tryptase constitutes a diagnostic criterion of this condition, correlates with its severity, and is useful in monitoring its outcome. Lange et al. examined a group of 100 children with cutaneous mastocytosis (CMC). The mean concentration of tryptase in this group was 10.17 ± 20.08 ng/mL, but differed depending on CMC form.³⁰ While the activity of the enzyme was 7.94 ± 15.62 ng/mL in the most frequently diagnosed papulonodular type of this condition, it was dramatically higher in the diffuse form, reaching 49.7 ± 42.64 ng/mL.

Conclusion

The mean serum concentration of tryptase in pediatric patients is 2.8 ± 2.2 ng/mL, with an upper reference limit of 7.2 ng/mL, which is lower than in adults. We postulate that the data presented here add to existing knowledge on the serum concentrations of tryptase in pediatric patients. However, further research is required, as the values documented in some age groups cannot be considered as definitive reference ranges. This requires a large sample of younger children to be examined.

Author Disclosure Statement

No competing financial interests exist.

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