Impact of Hymenoptera venom allergy and the effects of specific venom immunotherapy on mast cell metabolites in sensitized children*

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Abstract

Introduction and objective. Mast cells (MC) are effector cells during severe systemic reactions (SR) to Hymenoptera stings. Venom specific immunotherapy (VIT) is the treatment of choice for prevention of SR to stings. Tryptase and prostaglandin D$_2$ metabolites (PGD$_2$) are the markers of MC activation. The study design was to 1. compare baseline values of serum tryptase concentration (BST) and PGD$_2$ metabolites in children with/without venom sensitization, 2. to evaluate an influence of rush VIT on MC markers in treated children.

Materials and methods. Sensitized group: 25 children with SR to Hymenoptera sting. Control group: 19 healthy children. Active treatment: 5-day-rush-VIT. BST was evaluated by ImmunoCAP, PGD$_2$ metabolites in blood and urine by GC-NICI-MS.

Results. The baseline blood levels of MC markers were significantly higher, while urinary concentration of 9α,11β-PGF$_2$ was significantly lower in the whole group of venom-sensitized children compared to controls. Severity of SR showed negative correlation with urinary PGD$_2$ metabolites, while positive with plasma 9α,11β-PGF$_2$ and BST concentration. The highest sensitivity was obtained for plasma 9α,11β-PGF$_2$ whereas the highest specificity for urinary PGD-M.

Conclusions. In children with IgE-mediated SR to Hymenoptera stings, elevation of baseline values of PGD$_2$ metabolites in blood is accompanied by decreased excretion of its urinary metabolites. Assessment of stable PGD$_2$ metabolites might serve as an independent MC marker to identify allergic children. There is an association between urinary PGD$_2$ metabolites and severity of the SR to Hymenoptera stings.

Key words

cell, prostaglandin D$_2$/secretion, tryptases/blood, bee venom/therapeutic use, wasp venom/therapeutic use, desensitization, immunologic/methods

INTRODUCTION

Venom allergy is an example of an acute hypersensitivity reaction, a type of systemic reaction that occurs in less than 1% of the children population, and which are usually less severe than in adults. In children with the history of life-threatening venom-triggered systemic IgE-mediated reactions, a 3- to 5-year course of subcutaneous specific venom immunotherapy (VIT) is a treatment of choice, even at age below 5 years, providing 98% rate of long-lasting protection against a subsequent sting [1,2]. Most studies concerning risk factors and mechanism of both venom allergy and immunoprotection following treatment have been conducted in adults [3]. It is not possible to extrapolate directly these results to children [4].

Systemic reactions (SR) due to Hymenoptera stings are mainly initiated by the IgE-mediated release of mediators from mast cells and basophils upon exposure to venom allergens. Despite the progress in diagnostic methods of IgE-mediated mechanisms of sting allergy, effector cells of anaphylaxis still remain the target of many studies in venom allergy [5].

Demonstration of rapid, transient increase of serum tryptase level (enzymatically active mature β-tryptase) after specific allergen stimulation reflects massive mast cell activation and confirms the diagnosis of anaphylaxis [6] while baseline serum mast cell tryptase (BST) (inactive α,β-protryptases) concentration reflects the constitutive, individual mast cell load or activity. Values higher than 11.4 ng/mL are considered to be a marker of mast cell clonal disorders, including occult systemic mastocytosis [7]. Two recently published large cohort, multicenter studies allowed for the construction of a model/identification of the characteristic risk factors of severe systemic reaction after Hymenoptera field stings as well as during the buildup phase of VIT in adults [8, 9]. BST elevated above 5.84 ng/mL is among the identified predictors. There are some data on increase of BST with age, which might explain aggravated sting allergic reactions in elderly people [10]. Prostaglandin D$_2$ is a major cyclooxygenase product released by activated mast cells. It is unstable and metabolized by NADPH-dependent 11-ketoreductase to 9α,11β-PGF$_2$*, which is further transformed by oxidation to tetranor-PGD-M (PGD-M), both being rapidly excreted in urine [11, 12]. These early indicators of mast cell activation, were found to be useful in monitoring asthmatic adults [13, 14, 15] and children [16, 17]. Urinary concentration of 9α,11β-PGF$_2$*, proved to be a reliable marker of endogenous production of inflammatory mediators associated with anaphylaxis [18].
Objective. In our previous study we attempted to estimate the role of BST, as well as both blood and urinary prostaglandin D$_1$ metabolites, in predicting systemic side reaction during Hymenoptera venom immunotherapy in children [19]. In this study we wanted to compare the baseline values of prostaglandin D$_1$ metabolites and serum tryptase between symptomatic venom sensitized and healthy children, as well as to estimate the impact of rush schedule of venom immunotherapy on these parameters. There are no such studies in children.

MATERIALS AND METHOD

Study subjects. Sample of this prospective case-control study comprised two groups: a venom-sensitized group and a healthy control group. Venom-sensitized group included twenty five children (20 boys) aged 6–17 years (mean 11.5; SD 3.5) fulfilling both inclusion criteria of 1. Systemic (grade II-IV according to Mueller’s classification) reaction to Hymenoptera sting, children with the history of grade II of systemic reaction were qualified to VIT in case of increased exposure to stings (e.g. bee keepers families). 2. IgE-mediated venom allergy which was confirmed by positive result of at least one of the following procedures: a skin prick tests with venom extract concentration 100mcg/ml, intradermal tests with up-dosing to maximum concentration 1 mcg/ml, and specific serum IgE estimation by ImmunoCAP (Phadia AB, Uppsala, Sweden) were performed three to six weeks after systemic sting reaction. The venom extract used to skin testing as well as to treatment was obtained from the same manufacturer. Atopy was confirmed by positive skin prick test to inhalant allergens (Allergopharma Joachim Ganzer KG). After obtaining the results of diagnostic procedures venom-sensitized children were randomly divided into two groups of in-patient treatment: nineteen children (10 sensitized to Apis m. venom, 9 sensitized to Vespula spp. venom) to 5-day rush VIT with Venomenhal (HALAllergy, Sweden), and 6 children (sensitized to Apis m. venom) to 5-day rush VIT with Pharmalgen (ALK Abello, Denmark) (cumulative dose of venom up to 5-day rush VIT with Venomenhal (HALAllergy, negligible. There is a common source (Venomenhal (HALAllergy, Denmark) (cumulative dose of venom up to 5-day rush VIT with Pharmalgen (ALK Abello, Denmark) (cumulative dose)

An informed consent to participate in the study was obtained from parents of the study subjects. The study protocol was approved by Jagiellonian University Ethical Committee.

COLLECTION OF SAMPLES

In the treated group, samples of fasting venous blood and morning urine were taken twice for estimation of the mast cell biomarkers: 1. at baseline, before first dose of rush VIT; 2. after the last injection of incremental dose – for 9α,11β-PGF$_2\alpha$, measurement blood was collected 5 minutes after injection of the venom extract, and one hour later for tryptase estimation. Urine sample was collected within 1–2 hours after the last injection. In the control group blood and urine samples were taken once to estimate basal serum tryptase level, plasma 9α,11β-PGF$_2\alpha$, and the first morning urine sample metabolite content. Time schedule of samples collection reflected metabolism of both mediators and was in line with other authors [12, 14].

Blood samples. For tryptase were allowed to clot, then centrifuged and serum stored at ~80°C. Fluoroenzym immunoassay method using CAP System (Phadia, Uppsala, Sweden), which estimates both pro-forms and mature β tryptase, was performed. Serum tryptase detection ranges from 1 to 200 ng/mL, while values below are considered normal 10 ng/mL. Anticoagulated blood samples (citrate) for 9α,11β-PGF$_2\alpha$, were immediately centrifuged at 3500 rpm for 10 minutes and 500 pg deuterium labelled prostaglandin F$_2\alpha$ ([$^2$H$_4$] PGF$_2\alpha$) as an internal deuterated standard (Cayman Chemicals, Ann Arbor, Mi, USA) was added to 1 ml plasma. To 0.2 ml of urine samples internal deuterated standards of prostaglandin F$_2\alpha$ ([$^2$H$_4$] PGF$_2\alpha$) and PGD-M ([$^2$H$_5$] tetranor-PGD-M) were added. This compensated for analytes lost during sample preparations. All samples were stored at ~80°C and assayed within one month. Measurement of 9α,11β-PGF2 and PGD-M were performed using gas chromatography negative-ion chemical ionization mass spectrometry (GC-NICI-MS) (model 5896 series II; Hewlett Packard,Palo Alto,CA,USA) as described elsewhere [12, 13, 21]. The diagnostic ions were 569 m/z for 9α,11β-PGF$_2\alpha$ and 573 m/z for internal standard, while for PGD-M m/z 489 and m/z 495, respectively, where m/z denotes a mass-to-charge ratio: the quantity formed by dividing the mass of an ion by the unified atomic mass unit and by its charge number (positive absolute value)[22]. For plasma samples the detection limit was 1 pg/mL, while for urinary samples 0.5 ng/mg creatinine. The concentration for plasma samples was expressed in picogram per milliliter, for urinary samples in nanogram per milligram of creatinine.

Statistical analysis. Descriptive statistics were expressed as median (Me) and quartiles (Q1-Q3). Comparison of variables measured at two time points was done with Wilcoxon signed-rank test. For variables measured at the same time point comparison between two groups was done with Mann-Whitney test, while for more than two groups with Kruskal-Wallis test. Strength of relationship between variables was
estimated with Kendall tau-b coefficient. General Linear Model (GLM) was used to adjust the influence of age on PGD-M level in relationship to other parameters. The predictive value of PGD-M metabolites and BST for venom allergy was estimated using a receiver operator characteristics (ROC) curve for each PGD-M metabolite and BST separately. The cut point for the best sensitivity-to-specificity ratio was reported [23]. An area under the ROC curve (AUC) which is not significantly different from 0.5 means that prediction of venom allergy using the marker is no better than a result due to chance. As the study was not of cross-sectional design, the positive (or negative) predictive values, defined as percentages of cases among positive results of test (or percentage of non-cases among negative results of test, respectively), were computed based on assumption that prevalence of venom allergy in general children population equals 0.5 (different authors report values ranging from 0.3 to 0.8) using formulas depending on sensitivity and specificity of the markers [24, 25, 26]. Differences were regarded statistically significant at 0.05 type I error level. Analysis were done using SPSS 15 for Windows.

RESULTS

Characteristics of the treated group. The patients’ mean age was higher in *Apis m.*-venom sensitized children. In *Vespula* *spp.* sensitized children no one had the history of anaphylactic shock after a sting. Four patients were atopic, two with clinical manifestation of controlled mild bronchial asthma, in two symptoms of perennial allergic rhinitis were present. Regarding standard diagnostic procedures total IgE level was significantly higher in venom allergic children in comparison to controls (p=0.001). In children sensitized to *Apis m.*, the absolute values of venom specific IgE were higher than values of specific IgE in those sensitized to *Vespula* *spp.* (Tab. 1).

Baseline prostaglandin D2 metabolites and tryptase in children sensitized to Hymenoptera venom and control group. Gender and atopy did not influence the baseline level of mast cell biomarkers, while age showed significant negative correlation with urinary PGD-M at baseline (tau-b=−0.40, p=0.007).

In the whole group of children sensitized to *Hymenoptera* the baseline values of both mast cell markers in blood were significantly higher (p=0.025 for tryptase, and p<0.001 for 9α,11β-PGF2α, respectively), while baseline urinary concentration of 9α,11β-PGF2α significantly lower (p<0.001) in comparison to the control group (Tab. 2).

Regarding the type of sensitization, the baseline urinary PGD-M concentration was the highest in the control group, while the lowest in *Apis m.*-sensitized group (p=0.011). The serum tryptase level did not differ in this comparison (Fig. 1).

After adjustment for age, the difference in PGD-M urinary level between treated groups and controls was still significant (p=0.049). Both plasma and urinary concentration of 9α,11β-PGF2α retained their significance of difference (p=0.001 and p=0.002, respectively) as well.

A comparison within venom allergic children revealed higher urinary PGD-M concentration in *Vespula* sensitized than in *Apis m.* sensitized children (p=0.02) (Tab. 2). After adjustment for age, the difference lost its significance (p=0.101).

For the whole group of venom allergic children Mueller’s grade of systemic reaction after sting showed negative correlation with both urinary 9α,11β-PGF2α, and PGD-M concentrations at baseline. The median concentration of urinary PGD-M significantly decreased with the increase of Mueller’s grade score (from 5.21 (3.94–10.23) ng/ml creatine in the patients with grade II, to 2.12 (0.73–4.97) for the patients with grade III, and to 1.33 (0.43–2.86) for the patients with grade IV) (p=0.043). After adjustment for age, relationship between Mueller’s grade score and PGD-M concentration was still significant (p=0.001). Regarding the type of sensitization, it was still significant in *Vespula* sensitized group (p=0.035), while it lost its significance in *Apis m.* group (p=0.087) (Fig 2).

The median value of urinary 9α,11β-PGF2α concentration was the highest (1.60 (1.10–3.19)) in patients with grade II reaction, the lowest (0.58 (0.34–1.10)) in patients with the history of grade III sting reaction, while intermediate (0.65 (0.57–2.39)) in patients with the history of anaphylactic shock (grade IV) (p=0.012). There was no correlation between Mueller’s grade score and any mast cell mediators in plasma. A correlation coefficient between baseline both plasma and urinary 9α,11β-PGF2α concentration was still significant (p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=−0.50, p=0.034) in the whole group of allergic children.

Effects of venom immunotherapy on prostaglandin D2 metabolites and serum tryptase in sensitized children. VIT caused a significant increase of serum tryptase, but not in PGD-M metabolites in the whole treated group. Further analysis revealed, however, that increase of serum tryptase was significant for whole treated group (from 3.23 (2.41–5.06) to 3.65 (2.96–4.97) ng/mL; p=0.003) and *Apis m.* allergic children (from 3.34 (2.26–4.59) to 3.88 (2.99–4.83) ng/mL; p=0.010) (Fig. 1).

Table 1. Characteristics of VIT treated and control groups

|                  | *Apis m.* VIT group | Vespid VIT group | control group | Total   |
|------------------|---------------------|------------------|---------------|---------|
| N                | 16                  | 9                 | 19            | 44      |
| Gender: boys (%) | 12 (75%)            | 8 (89%)           | 8 (42%)       | 28 (64%)|
| Age: mean ± SD   | 12.4 ±3.0           | 9.9 ±3.9          | 9±1.5         | 10.4±3.1|
| Mueller’s classification grade |               |                  |               |         |
| II               | 7 (44%)             | 4 (44%)           | 0             | 11 (44%)|
| III              | 6 (38%)             | 5 (56%)           | 0             | 11 (44%)|
| IV               | 3 (18%)             | 0                 | 3 (12%)       | 6 (16%) |
| Presence of atopy| 3 (18%)             | 1 (11%)           | 0             | 4 (16%) |
| total IgE: Me (Q1-Q3) (kU/L) | 137.5 (67.2–229.0) | 91.8 (1.5–307.5) | 16.4 (8.1–30.5) |         |
| Vespid serum venom specific IgE: Me (Q1-Q3) (kU/L) | 0.5 (0.0–1.1) | 2.3 (1.1–18.1) | 0.00          |          |
| *Apis m.* serum venom specific IgE: Me (Q1-Q3) (kU/L) | 21.1 (8.5–46.9) | 0.0 (0.0–0.4) | 0.00          |          |
| Positive results of IDT | 11 (69%)             | 5 (56%)           | 0             | 16 (36%)|
| Positive results of SPT | 0 (0%)               | 0 (0%)            | 0             | 0 (0%)  |

IDT – intradermal tests, NP – not performed, SPT – skin prick tests, Me – median value, Q1 – 25th percentile, Q3 – 75th percentile.
Figure 1. Change in mast cell markers concentration during induction phase of VIT with reference to type of venom sensitization, in comparison to healthy controls.

Figure 2. Relationship between Mueller's grade and mast cell markers concentration with reference to type of venom sensitization.

Figure 3. Receiver-operating (ROC) characteristics for blood and urinary mast cell markers.
Table 2. Comparison of baseline median values (and interquartile ranges) of analyzed parameters between whole venom allergic group, regarding the type of venom sensitivity and control group

| Group                                      | Median (IQR)       | Control group 2.18 (1.75–3.29) | 13.32 (4.70–28.60) | 2.50 (1.74–3.47) | 6.31 (3.08–9.26) |
|--------------------------------------------|--------------------|----------------------------------|--------------------|------------------|------------------|
| Vespula spp. allergic group                | 3.21 (2.43–8.03)   | 23.72 (14.03–47.85)              | 0.88 (0.37–2.20)   | 4.97 (4.26–9.68) |
| Apis melifera allergic group               | 3.34 (2.26–4.59)   | 52.52 (27.28–70.05)              | 1.10 (0.57–1.60)   | 1.54 (0.72–5.17) |
| Whole group of venom allergic children     | 3.23 (2.41–5.06)   | 37.70 (22.52–62.00)              | 1.10 (0.54–2.12)   | 3.94 (1.26–6.61) |
| Control group                              | 2.18 (1.75–3.29)   | 13.32 (4.70–28.60)               | 2.50 (1.74–3.47)   | 6.31 (3.08–9.26) |

The relative increase of serum tryptase concentration in reference to the baseline value (relative delta bound) ranged from 88% to 158%. In four patients a relative increase ≥ 135% of the BST was observed, in one patient it was close to this bound. In spite that fact, immunotherapy was well tolerated, none of treated patients demonstrated systemic side effects during 5-day rush VIT.

There was no significant difference between baseline and post treatment VIT values in PGD, metabolite concentration. However, regardless the type of venom sensitization both plasma and urinary median concentration decreased, while PGD-M median concentration increased during induction phase treatment (Fig. 1).

Serum tryptase at baseline, correlated positively with its level after treatment in whole treated group (tau-b=0.79, p<0.001), and in subgroups analyzed for the type of sensitization (tau-b=0.78, p=0.004 and tau-b=0.83, p<0.001 in Vespula spp and Apis m. sensitized group, respectively). Urinary 9α,11β-PGF2 concentration after treatment correlated positively with its basal level only in Apis m. venom treated children (tau-b=0.48, p<0.001), while PGD-M pre/after VIT concentration correlated negatively in Vespula spp treated children (tau-b=-0.56, p=0.037).

Predictive properties of the markers to distinguish venom allergic children from controls. Baseline concentrations of both blood markers and urine 9α,11β-PGF2, evaluated using ROC curve, revealed the potency to discriminate between venom allergic children and controls (Figure 3). The area under the curve for those three markers reached 0.70, being significantly different from value of 0.5, what reflects probability of random assignment of patient as allergic. The highest value of AUC and the optimal sensitivity/ specificity ratio (for cut point of 1.67 ng/mg creatinine) in case of urinary 9α,11β-PGF2 was achieved. Positive predictive values estimated for analyzed markers were always higher than 1.5% reaching 1.0% for tryptase and plasma 9α,11β-PGF2. Negative predictive values were estimated as higher than 99.6% reaching 99.9% for both plasma and urine 9α,11β-PGF2 (Tab. 3).

DISCUSSION

The reasons why some cases develop allergic reactions from bee and wasp stings, while others do not, still remains a mystery. An answer to this question is being searched for from among a variety of possible influences, such as environmental factors on allergy, as well as in more sophisticated fields, like genetic aspects of venom allergy or evaluation of effector cells (mast cells and basophils) markers [27, 28, 29]. Our case-control study represents the first – and novel – attempt to compare the baseline values of prostaglandin D2 metabolites and serum tryptase between symptomatic venom sensitized and healthy children. We investigated also how 5-day venom specific rush VIT influences these parameters.

All *Hymenoptera* venom sensitized children were diagnosed in line with EAACI and AAAAI guidelines [1, 30, 31]. To achieve reliable laboratory results all drugs which may interfere with clinical symptoms (NSAID, LTRAs, antihistamines and systemic glucocorticosteroids) were withdrawn [32].

We did not find differences in level of mast cell markers between genders in venom allergic children, what might be due to the predominance of boys in the study group, which was also reported by other authors [33, 34]. In our study, the age of children showed a negative correlation with urinary PGD-M levels, but not with the other mast cell mediators, which is similar to the results obtained by Kielbasa et al. in their study on eicosanoids measurements in exhaled breath condensate of asthmatic children after provocation test [16]. Considering the type of venom sensitization and adjustment for the age of subjects, especially 9α,11β-PGF2 concentration in both biological fluids behaved more stable than urinary PGD-M or serum tryptase concentration, thus indicating that 9α,11β-PGF2, a PGD, metabolite, might provide better discriminating of venom allergic children from controls.

In venom sensitized children none of mast cell mediators measured in blood at baseline had discriminating power.

Table 3. Utility of the mast cell markers in indicating venom hypersensitivity

|                | Baseline Ac | Plasma Ac | Urinary Ac | Urinary PGD-M Ac |
|----------------|-------------|-----------|------------|------------------|
| AUC            | 0.70        | 0.80      | 0.81       | 0.65             |
| p for difference AUC and 0.5 | 0.025 | 0.001 | <0.001 | 0.095 |
| Sensitivity    | 0.64        | 0.88      | 0.79       | 0.58             |
| Specificity    | 0.68        | 0.58      | 0.72       | 0.76             |
| Cut point      | 2.57        | 15.57     | 1.67       | 6.06             |
| Unit of measure | ng/mL   | ng/mL    | ng/mg creatinine | ng/mg creatinine |
| Positive predictive value | 1.0 | 1.0 | 1.4 | 1.2 |
| Negative predictive value | 99.7 | 99.9 | 99.9 | 99.7 |

|                | Baseline Ac | Plasma Ac | Urinary Ac | Urinary PGD-M Ac |
|----------------|-------------|-----------|------------|------------------|
| AUC            | 0.70        | 0.80      | 0.81       | 0.65             |
| p for difference AUC and 0.5 | 0.025 | 0.001 | <0.001 | 0.095 |
| Sensitivity    | 0.64        | 0.88      | 0.79       | 0.58             |
| Specificity    | 0.68        | 0.58      | 0.72       | 0.76             |
| Cut point      | 2.57        | 15.57     | 1.67       | 6.06             |
| Unit of measure | ng/mL   | ng/mL    | ng/mg creatinine | ng/mg creatinine |
| Positive predictive value | 1.0 | 1.0 | 1.4 | 1.2 |
| Negative predictive value | 99.7 | 99.9 | 99.9 | 99.7 |
to distinguish between the type of sensitizing venom or severity of past anaphylactic reaction. An observation of the lower blood concentration of mast cell mediators in venom allergic children, as well as, the higher concentration in urine in control group, is consistent with the measurements results in which both urinary PGD_2 metabolites revealed the negative association with Mueller’s grade in case of whole group of venom allergic children, as well as separately, in _Vespula spp._ allergic children. Hence, indicating that higher baseline values of urinary PGD_2 metabolites can discriminate not only sensitized children from healthy ones, but also might be useful in indicating those with milder systemic reaction. It is in line with the results of our previous study, in which we described the higher urinary 9α,11β-PGF_2 concentration in children without severe SR to VIT in comparison to patients with severe SR [19]. We speculate that higher urinary excretion may reflect some protective mechanisms. The similar phenomenon – of higher PGD_2 metabolites values in milder symptoms – was observed by Bochenek et al. in asthmatic patients stratified by severity of disease [14]. It is tempting to speculate that in severe systemic reactions to _Hymenoptera_ stings, catabolism of PGD_2 to its inactive metabolites does not accelerate in response to its higher systemic biosynthesis.

We observed significant difference in baseline values of serum tryptase between venom allergic children and controls (3.23 ng/mL vs. 2.18 ng/mL). The similar data were reported by Yavuz in the age and sex-matched sample of Turkish children with insect venom allergy, showing that median level of BST in children with insect sting systemic reaction was significantly higher than in children with large local reaction and healthy subjects (4.2 vs 3.1 vs. 2.9 ng/mL, respectively) [35]. On the other hand in Komarow’s paper on 197 children (age range: 6 months to 18 years) there was no statistically significant difference between non-atopic and atopic subjects (3.44 vs 3.56 ng/mL); though venom allergy was not included into study group [36].

During 5-day rush venom specific immunotherapy with aqueous venom extract none of patients demonstrated systemic side effects (non-reactors). In our study, the median value of BST were equal to 3.21 ng/mL for _Vespula spp._ allergic group and to 3.34 ng/mL for _Apis m_ allergic group, which are below the median value 4.25 ng/mL from the cohort multicenter study, in which odds ratio of severe systemic reaction after sting in adults increased markedly when BST concentration was higher than 5 ng/mL [8]. In our recent study, we noted that children sensitized to _Apis m._ venom with mean value of serum tryptase level exceeding 7.75 ng/mL were at risk for systemic reaction to venom injection during rush protocol [19]. Other authors also reported that total tryptase level could indicate a risk for systemic reactions to rush immunotherapy and mast cell activation [37, 38]. Estimating the response to VIT, in comparison pre/post rush VIT values of mast cell mediators, only serum tryptase level, increased significantly, though within normal range, in the whole treated group and in _Apis m._ allergic subgroup. This is in line with the results pointing out that especially incremental phase of immunotherapy with bee venom extract goes together with the higher risk of systemic side effects and so this phase of treatment requires special attention [39]. Some authors pointed to an association between hypersensitivity reaction in adult venom allergic patients and a relative increase of serum tryptase level to ≥ 135% of the baseline value [40]. In our study, only four of twenty five patients had this parameter exceed that limit. As none of our patients had reaction to venom extract, data from more patients will be necessary to validate this finding. The schedule of samples collection in our study did not allow us to estimate the circadian rhythm of mast cells mediators concentration in children [41].

In contrast to BST compared during pre/post rush VIT, PGD_2 metabolites concentrations were stable, with non-significant decrease of 9α,11β-PGF, both plasma and urinary concentration and non-significant increase of PGD-M, which may reflect observed safety of both rush-VIT protocol and venom extract used in treatment. In addition to the abundant PGD-M, a tetranor end product of oxidation, used in our study [42], a new eicosanoid-derived urinary biomarker 2,3-dinor-9α,11β-PGF, emerged as the useful for monitoring symptomatic anaphylaxis and aspirin–intolerant asthma [43, 44]. Authors suggested that it might be the most reliable indicator in determining PGD_2 production in vivo, thus indicating that PGD_2 metabolites are mediators of special interest in studies dedicated to allergy.

At baseline, the plasma levels of PGD_2 metabolite were elevated, while urinary PGD_2 metabolites were decreased, discriminating venom allergic children from control group. Analysis of ROC curve properties showed acceptable parameters for all markers except urinary PGD-M, for which areas under curve were significantly higher than value of 0.5, reflecting probability of random assignment of the patient as venom-allergic or not. Among these three parameters, however, only for plasma 9α,11β-PGF the ROC curve does not intersects the reference line, indicating it as even better marker of venom hypersensitivity than BST. This suggests that for a very low level of tryptase and for a very high level of urinary 9α,11β-PGF, these markers may wrongly classify healthy children as venom allergic ones, and vice versa. This may be due to the fact that control group in this study was smaller in size than the allergic one, what may underrepresent number of healthy children with the lowest values of BST. This might lead to situation that the smallest level of tryptase were observed in allergic children, although when comparing allergic and control group, the second one had significantly lower level of BST. Extended number of subjects in future studies are necessary to validate here reported observations. Though mast cells are the main effector cells of anaphylaxis in comparison to basophils, an attempts to evaluate venom allergy with basophil markers, are much more advanced in comparison to PGD_2 metabolites, originating exclusively from mast cells. Basophil activation test (BAT) has been introduced in both diagnosis and VIT monitoring of venom allergic patients [45, 46, 47, 48]. There are also attempts to introduce it as an useful diagnostic tool in IgE-negative patients with the history of systemic sting reaction [49]. The first, very interesting data on blood basophils as a target for VIT, indicated for impaired release of both preformed and newly generated mediators during 3.5-hours lasting ultra-rush VIT protocol [50]. Just recently preliminary data on simultaneous evaluation of BAT and mast cell markers during first 40 days of VIT introduction (build-up phase and two maintenance doses) was published recently. Authors concluded that plasma levels of 9α,11β-PGF2 decrease while numbers of activated basophils increase during the initial phase of bee venom rush immunotherapy in children [51].
CONCLUSIONS

Children with history of IgE-mediated systemic reaction to *Hymenoptera* stings are characterized by elevated baseline values of mast cell metabolites in blood, and by decreased urinary PGD stable metabolite concentration, that discriminates them from non-allergic children. Thus, assessment of the stable urinary PGD metabolite might serve as an independent marker of mast cell activation in these patients. There is a negative association between urinary PGD metabolites and severity of the systemic reactions. Uneventful rush venom therapy is not associated with any changes in either plasma or urinary levels of PGD stable metabolites.

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