Original Article

Diagnostic Value of Tryptase in Food Allergic Reactions: A Prospective Study of 160 Adult Peanut Challenges

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What is already known about this topic? Serum tryptase has been shown to be useful in confirming anaphylaxis caused by venom or drugs; however, its utility in food allergic reactions is unknown.

What does this article add to our knowledge? This prospective study shows that tryptase rises acutely compared with baseline levels during peanut allergic reactions. Peak levels occur at 2 hours and correlate with severity. An optimal cutoff in tryptase rise of 30% is established which identifies an allergic reaction.

How does this study impact current management guidelines? Serum tryptase measurement is valuable in food allergic reactions. The cutoff in tryptase rise may guide clinicians in establishing whether mast cell activation in a food allergic reaction has occurred in both an emergency and challenge setting.

BACKGROUND: Serum tryptase is useful in diagnosing drug and venom anaphylaxis. Its utility in food anaphylaxis is unknown.
OBJECTIVE: The objective of this study was to determine whether tryptase rises in food allergic reactions, optimal sampling time points, and a diagnostic cutoff for confirming a clinical reaction.
METHODS: Characterized peanut allergic patients were recruited and underwent up to 4 peanut challenges and 1 placebo challenge each. Tryptase was measured serially on challenge days both before (baseline) and during the challenge. The peak percentage tryptase rise (peak/baseline) was related to reaction severity. Receiver operating characteristic (ROC) curves were generated establishing an optimal diagnostic cutoff.
RESULTS: Tryptase was analyzed in 160 reactive (9% anaphylaxis) and 45 nonreactive (placebo) challenges in 50 adults aged 18 to 39 years. Tryptase rose above the normal range (11.4 ng/mL) in 4 of 160 reactions. When compared with baseline levels, a rise was observed in 100 of 160 (62.5%) reactions and 0 of 45 placebo challenges. The median rise (95% confidence interval [CI]) for all reactions was 25% (13.3% to 33.3%) and 70.8% (33.3% to 300%) during anaphylaxis. Peak levels occurred at 2 hours and correlated with severity ($P < .05$). Moderate-to-severe respiratory symptoms, generalized erythema, dizziness, and hypotension were correlated with a higher peak/baseline tryptase ($P < .05$). ROC curve analysis demonstrated the optimal cutoff to identify a reaction as a 30% rise (sensitivity 0.53; specificity 0.85), area under the curve 0.72 (95% CI, 0.67-0.78).
CONCLUSIONS: Serum tryptase measurement is valuable in food allergic reactions, and correlates with symptom severity. Comparing peak reaction levels at 2 hours with baseline is essential. A rise in tryptase of 30% is associated with food allergic reactions. **(J Allergy Clin Immunol Pract 2018;■:■-■)**

Key words: *Peanut allergy; Anaphylaxis; Adults; Tryptase; Diagnosis*

Serum tryptase is a marker of mast cell activation, and a rise in tryptase is a useful indicator of whether an allergic reaction has taken place. Tryptase has been shown to be of value in allergic reactions triggered by venom[1] and drugs[2]; however, its utility in food allergic reactions remains unknown. Some studies report that tryptase does not rise in food-induced anaphylaxis[3] nor in nonhypotensive reactions. These studies are largely based on data derived from postmortem samples of patients who died from anaphylaxis[4] or patients presenting acutely to emergency departments resulting in varying sample times and a bias toward more severe reactions. Experimental evidence of tryptase in food allergic reactions and in particular on its typical time course is lacking.

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Abbreviations used
CI- Confidence interval
IQR- Interquartile range
ROC- Receiver operating characteristic
SPT- Skin prick test

We present a large prospective study of the diagnostic utility of serum tryptase in experimentally induced peanut allergic reactions of varying severities. Our aim was to establish whether tryptase rises in food allergic reactions, the optimal time point for tryptase sampling, and an optimal diagnostic cutoff in serum tryptase rises for determining a reaction versus no reaction.

METHODS

We carried out a prospective study of mast cell tryptase during peanut allergic reactions.

Patients

Adult patients with a history of systemic reactions (urticaria, angioedema, respiratory/gastrointestinal tract symptoms, and anaphylaxis), with acute onset of symptoms after ingestion (up to 2 hours) of peanut, were recruited from 2013 to 2016. Allergic status was determined by evidence of sensitization to peanut demonstrated by positive skin prick tests (SPT) and positive serum specific IgE using ImmunoCAP (Thermofisher, Uppsala, Sweden) to peanut and peanut components Arah 2 and/or 1 and 3. A positive test was defined using the criteria of $\geq$3 mm for SPT and $\geq$0.35 kUA/L for ImmunoCAP. All patients underwent an initial peanut challenge. The patients were given incrementally increasing doses of peanut protein until they developed clear objective signs of an allergic reaction. They were given increasing doses of masked food matrix containing peanut flour. Doses were delivered at 30-minute intervals starting with an initial dose of 3 μg to a total cumulative dose of 1,433 mg of peanut protein. Reaction severity was scored according to the Ewan and Clark severity scale.[5] Anaphylaxis was defined as 2 or more of the following rapidly occurring symptoms (minutes to hours) after exposure to peanut: generalized involvement of the skin-mucosal tissue, respiratory compromise (pronounced dyspnoea, wheeze, bronchospasm), reduced blood pressure or associated symptoms, severe and persistent abdominal symptoms (cramping abdominal pain and vomiting),[6] and corresponded to Ewan and Clark severity grade 5 reactions. The first peanut challenge was double blind with all patients undertaking a placebo arm. In the placebo arm, patients were given the same masked food matrix without allergen. The initial challenge was followed by 3 further open challenges. Ethical approval was obtained from the National Research Ethics Service Committee East of England. Informed consent was obtained from all patients.

Mast cell tryptase

Blood samples for mast cell tryptase were taken on each challenge day before the commencement of peanut doses (prechallenge sample, at the onset of reaction, and at 1 and 2 hours after the reaction). On nonreactive challenge days, samples were taken before the commencement of doses and at 2 hours after the last dose. An initial serum tryptase was also taken for each patient on his or her screening visit. Tryptase was measured using UniCAP (Thermo Scientific, Uppsala, Sweden). The detection limit of the assay was 1 ng/mL. The upper limit of normal was taken to be 11.4 ng/mL (95th centile).

Statistical analysis

Mean and standard deviations were calculated, and because of nonnormality, median and a nonparametric confidence interval (CI) around the median were used. A P value of $<.05$ was classed as statistically significant. The peak percentage rise was calculated as follows: (peak tryptase level during reaction/same day prechallenge sample) $\times$ 100. Correlations were calculated. To investigate the utility of serum tryptase in determining whether an allergic reaction has occurred, receiver operating characteristic (ROC) curves were used to establish the cutoff providing the best sensitivity and specificity for the test. Statistical analyses were performed with STATA version 12 (StataCorp, College Station, Tex).

RESULTS

Patient characteristics

Fifty adults aged 18 to 39 years (median age 20.8, M = 28, F = 22) were included in the study. Tryptase readings were measured in 177 reactive peanut and 45 nonreactive challenges. In 17 of 177 challenges, tryptase levels were $<$1 ng/mL and therefore were unable to be analyzed. All patients were reactive and developed objective symptoms during the peanut challenges. Five patients developed mild, transient subjective symptoms during placebo challenges; however, no patient developed objective symptoms. Patients were therefore deemed nonreactive during the placebo challenges. Fourteen of the 160 reactions (9%) were classed as anaphylaxis. No patients had a raised basal tryptase level.

Tryptase during placebo challenges

Tryptase readings were available for 45 placebo challenges. In the placebo challenges, the percentage change of the 2-hour reading/baseline was zero in 21 cases, positive in 11, and negative in 13. The median peak percentage rise was zero (95% CI, 0, 0; interquartile range [IQR] −1.92, 4.31), the arithmetic mean (which gives higher weight to proportional increases than decreases) was 1.4%, and the geometric mean (which gives both proportional increases and decreases equal weight) was −0.1%. Patients with higher placebo rises did not exhibit higher reaction rises.

Tryptase rise during reactions

Tryptase was raised above the upper limit of the normal range (11.4 ng/mL) in 4 of 160 reactions, and all 4 reactions were severe anaphylaxis. The predominant symptom was hypotension in 1 of 4 reactions and profound dyspnoea with no hypotension in 3 of 4 reactions. Peak levels were 17, 13, 12.2, and 12 ng/mL, respectively (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). In 10 of 14 anaphylactic reactions, the peak tryptase remained in the normal range. The peak percentage rise was calculated for all reactions. Relative to the baseline, a rise was noted in 100 of 160 reactions (62.5%), anaphylaxis group 14 of 14 (100%), and nonanaphylaxis group 86 of 146 (59%). The average peak percentage rise for all reactions was 34.8% (median 25%, 95% CI, 13.3% to 33.3%). In the anaphylaxis group, the average rise was 148.4% (median 70.8%, 95% CI, 33.3% to 300%), and in the nonanaphylaxis group, 23.9% (median 14.0%, 95% CI, 0% to 25%).

Time course of serum tryptase during reactions

Median percentage change in tryptase over time is shown in Figure 1 (*A*, *B*). Relative to a completely flat time course in placebo challenges, tryptase levels rose over time in positive