Author's response to reviews

Title: Excretion of urinary histamine and N-tele methylhistamine in gastrointestinal food allergy

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Author's response to reviews: see over
Point-to-point reply to the reviewer’s report

Thank you for your interest in our research work and for giving further stimulating comments to improve our paper concerning urinary histamine and methylhistamine excretion in gastrointestinal mediated allergy (GMA)

Comments to the reviewer Ivy Shiue

1 The title was not supplemented with more specific details and is now “Excretion of urinary histamine and N-tele methylhistamine in patients with gastrointestinal food allergy compared to food-tolerant controls during an unrestricted diet and a hypoallergenic diet”

2 This a retrospective study type comparing a food-allergic group with healthy food-tolerant individuals as controls (case control study). The urinary mediator values and clinical diagnostic tests were collected prospectively, entered into a data base and retrospectively analysed. Its aim is now included in the abstract and more specifically described in the Introduction section.

4 The study design is now given in the Materials and Methods section (see patients) and in figure 1 illustrating the separation of patients with gastrointestinal food allergy from a large group of patients complaining functional food related symptoms, but not having evidence of manifest significant organpathological diseases, celiac disease or any infectious disease.

However, due to the complexity of food challenge procedures only a minority of patients wanted to undergo BPCFCs (56 of potential 153; 36.6%). But in these 56 patients we are sure to have precisely diagnosed GMA as outlined in several national and international food allergy guidelines.

Patient characteristics are now included in the results section.
As stated in the unrevised form of this paper at page 6 ethics approval and foundation are given (see “All patients gave their informed consent and the study protocol was approved by the local ethics committee (No. 2500) and supported, in part, by grants from Marohn foundation (Erlangen, Germany)”).

Table 1 describes the characteristics of the allergy group in its whole and in the first table for the electronic supplement the individual data of each allergy patients are consecutively listed. As suggested by this reviewer we included now a further table 2 in the paper exactly describing the characteristics of the control group.

As the main focus of this study was to evaluate firstly normal excretion parameters for healthy individuals, then to assess the diagnostic accuracy of urine histamine and -methylhistamine excretion for identifying gastrointestinal food allergy, we did not perform other statistical modeling beyond the given data.

Some language corrections have been made and typing errors were replaced.
Point-to-point reply to the reviewer’s report

Yuchi Adachi

Major compulsatory revisions

Histamine is not only secreted after type I allergy, but also after the other allergy types as mentioned in the literature. Type II allergy includes generation of C3a and C5a, which act as anaphylactic triggers for mast cells and basophils. Such effects have also been described as non-IgE component in food allergy of children with atopic dermatitis and may be also suspected in adults with GMA.


Type III allergy is characterized by immune complex formation of the IgG-, IgM-, IgA- and/or IgE-type which have been shown to activate mast cells, eosinophils and basophils and was also found in a few patients of our cohort (see also supplement for electrobic version, point 1).


Ghebrehiwet B, Kew RR, Gruber BL, Marchese MJ, Peerschke EI, Reid KB. Murine mast cells express two types of C1q receptors that are involved in the induction of chemotaxis and chemokinesis. J Immunol. 1995 Sep 1;155(5): 2614-9

In type IV allergy, it is well known that histamine releasing factors (HRFs, cytokines) contribute to an increased mast cell and basophil activation which may also contribute to increased tissue histamine concentrations with subsequent metabolization of histamine to methylhistamine which accumulates in urine. Thus, the type IV allergy and histamine production are worthwhile to be investigated in GMA as adults may have non-IgE allergy types.

Kawakami T, Kashiwakura J, Kawakami Y. Histamine-releasing factor and
immunoglobulins in asthma and allergy. Allergy Asthma Immunol Res. 2014 Jan;6(1):6-12

Jyonouchi H. Non-IgE mediated food allergy. Inflamm Allergy Drug Targets 2008; 7(3): 173 – 180


As suggested by this reviewer we were able to analyze the data for type I food allergy and the non-IgE mediated forms. This data have now been included as a further table 3 and a new results section in the paper and show similar results as reported for the whole GMA group. Interestingly, patients with non-IgE GMA were also found to have increased UMH values as we have observed earlier and suggested in the above listed literature findings.

As indicated in the Materials and Methods section (original version page 8, urinary mediator excretion) the unrestricted diet included ingestion of staple foods as patients consume it on a daily basis at home when suffering from symptoms. The staple foods (as given for skin prick testing and food-specific IgE), were consumed, at least once, either at day 1 and/or day 2, to encompass all common food groups. Among these staple foods following qualitative groups were allowed along with sugar and salt: Beef, egg, fish, fruit, pork, wheat, rye, soy, bran, milk, cheese, nuts, barley, coffee, oats, maize, apple, peach, banana and vegetables, but quantitative amounts were not recorded for practical reasons. Extraordinary food allergens like kiwi, Asiatic spices, curry, sesame etc were not allowed as well as alcoholic beverages. These information were now included in the Materials and Methods section (urinary mediator excretion, page 9).

As indicated in the Materials and Methods section (original version page 8, urinary mediator excretion) the patients underwent the functional diet test before confirmation of GMA by BPCFCs (see “During differential diagnostic work up all patients (without any medication) underwent a functional diet test during two subsequent days with unrestricted diet (day 1 and 2) and two further days with potato-rice diet (day 3 and
4). This is now more clearly given in the same section and was also indicated in the section “Diagnosis of GMA” (see also “Food challenges were only performed when patients had a clear resolution of their symptoms under the hypoallergenic diet with improvement of their symptom scores < 3 points.”).

Minor essential revisions

As UH and UMH measurements runs in our hospital on a routine basis, urine samples were checked for pH. In the case of pH>3, this might indicate false urine preparation, possibly influenced by bacterial histamine production. Thus, such urine samples with ph > 3 were not used for further analysis.

This is now given in the results section with 3/112 samples in the GMA group (2.7%) under potato-rice diet which did not fulfil above mentioned ph criteria. All other groups and diet types had normally processed urine samples with ph<3.

From each patient two UH and two UMH levels (day 1 and 2) were obtained for each diet type. These values were entered into the data base for each diet type and analysed correspondingly for the disease group and diet type. Intraindividual variations of both groups were analysed and given in the results section.

There may be several reasons why the symptom score did not correlate significantly with UH or UMH. As we have admitted in the paper, there may be several other mediators causing symptoms in patients with GMA (platelet activating factor, eicosanoids, tryptase etc) or open-labelling of the study. However, we gave the real statistical analysis and this points to the fact that histamine production is obviously not the single dominating mediator in GMA. However, this has also been reported in anaphylaxis and appears to be mainly attributed to the great plethora of mediators contained in mast cells, basophils and eosinophils.

The spelling mistakes have now been corrected.