Measurement of diaminoxidase (DAO) during low-histamine or ordinary diet in patients with and without histamine intolerance

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Abstract

Quantification of diamine oxidase (DAO) concentrations within the serum has been proposed as an adjunctive diagnostic modality for the evaluation of histamine intolerance (HIT). Limited empirical data exist concerning the influence of dietary patterns on DAO levels. In the context of a prospective study employing a crossover design, 18 individuals diagnosed with HIT were randomized to initiate either a low histamine diet (LHD) or a conventional mixed diet (MXD). Serum DAO concentrations were measured at the commencement of the study and following each dietary phase. A control group underwent analogous DAO assessments without imposition of dietary constraints.

Throughout the period of histamine-restricted dietary intervention, discernible distinctions in alterations of DAO levels failed to manifest in comparison to alterations observed during the MXD phase. Specifically, among the cohort, 10 of the 18 patients exhibited elevated DAO values subsequent to the LHD regimen, while the remaining eight displayed either reduced or unchanging DAO levels. The prevalence of elevated DAO levels in the LHD group did not differ significantly from that observed in the control group during the MXD phase. Additionally, during the LHD phase, patients reported a significant reduction in gastrointestinal and cutaneous symptoms.

This prospective investigation underscores the enduring utility of a histamine-restricted diet, coupled with structured dietary reintroduction, as an efficacious diagnostic approach for individuals presenting with suspected food-related histamine hypersensitivity. Notably, measurement of DAO levels appears to furnish only a limited capacity to discern dietary-induced fluctuations. Notwithstanding, the dynamics of DAO alteration do not appear to exhibit a discernible association with specific dietary patterns, a finding consistent across both patient and control groups.

Introduction

Histamine is a bioactive amine which is synthesized through the decarboxylation process of its precursor, histidine, which is an essential amino acid. The European Food Safety Authority (EFSA) states that bioactive amines presented in foods can pose a health risk to consumers (1). In the context of histamine, it is commonly associated with adverse reactions categorized as non-allergic food hypersensitivity, as classified by the World Allergy Organization (2). The prevalence of histamine hypersensitivity as a non-IgE mediated subtype of hypersensitivity is currently unknown, but it is estimated to affect approximately 1% of the general population, with a higher incidence among middle-aged individuals (3). Nevertheless, it is important to note that the actual impact of histamine hypersensitivity could be more substantial than the current estimations suggested (4). Over the past decade, histamine hypersensitivity has gathered increased attention both socially and scientifically, resulting in a significant rise of publications in this field (5).

Histamine is found in various foods such as meat, cheese, fish, and alcoholic beverages. The concentration of histamine content in these foods varies, as it increases with maturation and bacterial
contamination. Therefore, determining the amine content in specific foods is not relevant since the concentration may differ between the different products (3). The impact of combining foods with varying concentrations of different amines in patients with histamine hypersensitivity is currently unknown.

Histamine, whether it is arising endogenously or exogenously from food, it is metabolized through two known degradation pathways: methylation by histamine-N-methyltransferase (HNMT) and oxidative degradation by diamine oxidase (DAO) (5). DAO enzymatic activity can be measured in serum samples, intestinal mucosa and in stools (6). The determination of DAO levels and the interpretation of results have been contradictory in some published studies, leading to ongoing interest in studying whether symptoms correlate with the measured DAO levels. In a study of adult patients with symptoms related to histamine intolerance, the DAO levels in the blood were significantly lower compared to a control group (7). A small retrospective study found lower DAO levels in the blood in histamine intolerant subjects compared to healthy volunteers (8) Another retrospective study concluded that the diagnosis of histamine intolerance could be based on low DAO levels and more severe symptoms, although this study lacked a control group (9).

The positive effect of histamine reduced diet on patients with chronic spontaneous urticaria but also in patients with chronic eczema and asthma has been sporadically reported previously in some short studies (10–14). An overlap between gastrointestinal symptoms and relation to intake of histamine rich foods has also been elucidated (15). The influence of histamine free or histamine reduced diet on serum DAO levels has been studied to a limited extent but in patients with chronic idiopathic urticaria it was shown higher histamine plasma levels during the histamine free diet (16). A relation between gastrointestinal complaints due to irritable bowel syndrome (IBS) and the consumption of histamine-rich foods has been discerned, suggesting a potential association between histamine-rich foods and IBS (ref Böhn)Short-term effects on DAO levels, measured during a histamine-rich meal challenge, could not be detected among patients or healthy controls where changes in the DAO levels did not differ before and after the challenge (17). Additionally, the daily profiling of DAO serum activity showed reduced levels during the daytime in a subgroup of patients with histamine hypersensitivity compared to patients with IgE-mediated food allergies, although the DAO levels were not measured during any amine-free dietary interventions (18). Lackner et al. identified a group of patients with low DAO levels and retrospectively collected information on diet adherence and related symptoms. They found a significant increase in serum DAO levels among patients who strictly followed a diet, but the increase was also observed in the group without a specific diet regimen, and the long follow-up period increased the risk of non-adherence to the diet up to 13 months (19).

More data is needed to understand how DAO activity levels are affected by a MXD diet and a diet with low histamine content. The aim of this study was to assess DAO activity measured in serum in relation to diet (mixed compared to histamine-reduced diet) and to evaluate self-assessed food-related symptoms associated with histamine hypersensitivity during the dietary intervention period.

Methods
The study was conducted as a randomized controlled trial using a crossover design. A total of 18 individuals over the age of 18 were referred to the Department of Allergology at Sahlgrenska University Hospital in Gothenburg for investigation of suspected histamine intolerance (HIT). Between 2018 and 2022, a total of 27 participants were eligible for inclusion in the study. All 18 patients had been diagnosed with histamine intolerance by allergologist physician. IgE-mediated food allergy was excluded. The exclusion criteria for study invitation included adherence to a vegan or incompatible diet with the study protocol and diagnosed inflammatory or systemic rheumatologic disease.

The participants were requested to complete a questionnaire regarding atopic diseases, gastrointestinal symptoms, medications, and menstrual cycle status (if applicable). A questionnaire developed and used in an earlier study (20) was adapted for this purpose.

The participants were randomly assigned to start with either a low-histamine diet (LHD) or an ordinary mixed diet (OMD) with a simple randomisation model (Fig. 1) and were instructed to follow the assigned diet for a duration of 3 weeks. The LHD involved the reduction of foods and beverages known to be high in histamine content, as determined by the European Food Safety Authority (EFSA) report and implemented by the Swedish Food Agency. During the ordinary mixed diet (OMD) phase, patients were advised to consume at least two food items per day that contained higher amounts of histamine and otherwise to adhere to a mixed diet regime. Throughout the study, all patients maintained regular contact with the study dietitian to ensure adherence to the diet and protocol.

Participants recorded various food-related symptoms in a symptom diary using a previously validated scale from another study (21). Symptoms were rated on a scale of 0 to 3, with 0 indicating no symptoms and 3 indicating severe symptoms. The recorded symptom categories included pain (gastrointestinal pain), skin symptoms (flush, itching, rash, urticaria), headache, number of stools per day, and altered stool consistency (such as loose stool or diarrhoea). Participants were allowed to manually add any additional symptoms not covered by the symptom diary in open questions.

Measurement of diamine oxidase (DAO) activity in serum was performed at the beginning of the study, after 3 weeks of diet intervention, and again after another 3 weeks (Fig. 1) Patients did not to use additional medication with antihistamines, glucocorticoids or anti-inflammatory drugs four week prior and under the dietary intervention. Additionally, the use of DAO enzyme supplements during the study period was not permitted and the participants were not under any medication with drugs that could block the DAO activity reported in the literature (22, 23)

The control group, consisting of 10 subjects without any reported histamine-related food hypersensitivity, underwent the same procedure with analysis of the DAO levels in serum and were asked to complete the same questionnaires. No dietary restrictions were imposed on the control group, and they were instructed to maintain their regular diet.

Measurement of DAO
The measurement of DAO levels in the samples was performed using a commercially available kit (DAO-REA Sciotec, HS 421 - 37; Tulln an der Donau, Austria) according to the manufacturer's instructions. (24)

Statistics

Statistical analyses were conducted using SAS 9.4 from SAS Institute Inc., Cary. For continuous variables of DAO, means and standard deviations (SD) are presented. DAO values were log-transformed to reduce skewness. Categorical variables are expressed as numbers and percentages. Differences between groups were assessed using Friedman's test and GLM/Mixed models. Symptom analysis and corresponding box plots were generated using R Studio Version 1.3.959. The analysis was based on aggregated symptom data, where daily values for each symptom (stomach pain, skin symptoms, headache) were summed for each diet (low histamine diet and mixed diet) to yield a single value per diet per individual. Daily data values for the outcome "stools" were similarly summed for each symptom and diet, and comparisons were made using a paired t-test. Statistical significance was set at p < 0.05 for all comparisons.

The study was approved from the ethics review authority in Gothenburg, Sweden (Document no. T351-17).

Results

During the observational period spanning from 2018 to 2022, a total of 27 patients met the eligibility criteria for participation, and subsequently, 20 patients consented to participate in the study. Notably, two patients withdrew from the study for distinct reasons: one due to intolerable adverse reactions encountered during the mixed diet (MXD) phase and another due to unrelated issues.

Within the patient cohort, a subgroup of 12 out of 18 individuals presented with atopic conditions, encompassing six cases of allergic rhinoconjunctivitis. Among this subgroup, three cases featured optimally managed TH2-high asthma, and one case presented with atopic eczema, while the remaining six patients exhibited no concurrent atopic conditions. Importantly, none of the recruited patients had preexisting gastrointestinal ailments, such as irritable bowel disease, celiac disease, non-celiac gluten sensitivity (NCGS), carbohydrate or lactose intolerance, inflammatory bowel disease, eosinophilic disorders, or systemic conditions including mast cell disorders or rheumatic disorders. Furthermore, none of the female patients in the study were pregnant.

Among the ten participants initially recruited for the control group, nine individuals met the inclusion criteria. Four of these individuals had allergic rhinoconjunctivitis but did not possess a medical history or clinical manifestations indicative of oral allergy syndrome (OAS) or food-related symptoms. Crucially, none of the control group subjects reported experiencing food-related hypersensitivity or associated symptoms (refer to Table 1a for details).
Table 1a

Descriptive data of patients and controls which participated in the study

<table>
<thead>
<tr>
<th></th>
<th>Patients n: 18</th>
<th>Control n: 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39 (26–69)</td>
<td>51 (22–53)</td>
</tr>
<tr>
<td>Gender, female /male</td>
<td>14/4</td>
<td>7/2</td>
</tr>
<tr>
<td>Any atopy reported</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>GI symptoms reported</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Asthma diagnosed</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1b: DAO values in the serum in both patients and controls during the different dietary interventions

<table>
<thead>
<tr>
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<th>Patients n: 18</th>
<th>Control n: 9</th>
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<td>12</td>
<td>4</td>
</tr>
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<td>GI symptoms reported</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Asthma diagnosed</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mixed diet for whole period

The DAO levels in relation to low histamine diet

The median baseline diamine oxidase (DAO) activity was 9.8 U/ml within the patient group and 12.3 U/ml within the control group (as presented in Table 1b). It is noteworthy that the intergroup disparity in DAO activity at baseline did not attain statistical significance.

An inclination toward elevated DAO activity levels following the low histamine diet (LHD) regimen was observed in 8 of the 18 patients, while the remaining 10 patients exhibited stable or diminished DAO levels after a 3-week period of LHD. Further analysis of DAO levels, encompassing assessments conducted prior to and during the dietary intervention under a crossover design (baseline, post-LHD, and post-mixed diet phases), revealed no statistically significant differences in DAO levels across distinct
phases and dietary interventions, as ascertained through non-parametric testing employing Friedman's analysis of variance (ANOVA), as illustrated in Fig. 2.

**Symptoms related to the histamine reduced diet**

To assess the symptoms related to the diets, daily data values for each symptom category (gastrointestinal pain, skin, headache) were aggregated for each diet (histamine-reduced and ordinary mixed diet) to generate a total symptom score per diet and per individual. These scores were compared using a paired t-test. The analyses revealed significant differences in the categories, specifically in gastrointestinal pain, but also in skin symptoms, and the total score for all symptoms between the histamine-reduced diet and the mixed diet. However, no significant differences were observed in the symptoms of headache or stool alternation (Fig. 3).

The control group did not report any symptoms during the ordinary and mixed diets (data not shown).

**Discussion**

Diamine oxidase (DAO has been subjected to analysis as a diagnostic modality for histamine intolerance, as evidenced by multiple studies (7, 8, 12). Nevertheless, divergent guidelines and uncertainties concerning its diagnostic efficacy have been prominently raised (9, 16, 25)). Prior research has suggested that long-term elimination diets are associated with the recovery of DAO levels in individuals reporting histamine intolerance (13). In our prospective study, no significant difference in DAO activity was observed based on the type of diet. Baseline DAO values exhibited a wide distribution, with approximately half of the participants having values below the suggested cut-off of 10 U/ml. Baseline DAO values in the control group did not differ significantly from those in the patient group, indicating that DAO cannot be utilized as a diagnostic tool.

When comparing DAO levels between different diets using a crossover interventional model, our findings indicate that the LHD applied for three weeks had no impact on the DAO levels, nor did the MXD. These results contradict the findings of a study conducted by Lackner *et al.*, which employed a similar diet model (19).

The analysis of subjectively self-reported symptoms throughout the study period revealed that patients experienced significantly more skin symptoms (flushing, itching, urticaria) and gastrointestinal pain when consuming a MXD which is in accordance with previous observations and publications (3, 26). However, no increase in the occurrence of headaches or other neurological symptoms was observed when shifting from LHD to MXD with histamine-rich foods.

The histamine-restricted diet appears to exhibit a positive impact on various chronic symptoms, some of which may be of a food-related nature. Previous observations have demonstrated a prominent amelioration of cutaneous manifestations in certain patients with atopic dermatitis following adherence to a Low Histamine Diet (LHD) (27). Additionally, literature indicates an association between the consumption of histamine-rich foods and refractory chronic urticaria, albeit with concurrent
pharmacological relief and improvement in urticaria symptoms during LHD. (28) Interestingly, it has been observed that individuals with chronic food-related gastrointestinal symptoms, carbohydrate malabsorption, and non-celiac gluten enteropathy may benefit from reduced histamine intake(29). This suggests a plausible link between the ingestion of elevated histamine levels in foods and the manifestation of localized intestinal enteropathy in the gastrointestinal tract, of non-immunologic origin (30).

In light of these findings, it is reasonable to assert that a diagnostic exploration of a dietary intervention with low histamine content should be considered for all patients presenting with chronic diffuse symptoms, particularly when clinical history suggests a potential association with food-related etiology. Further investigation is required to determine the optimal extension of a low-histamine diet (LHD), considering specific foods containing high or higher histamine content, or a combination of various biogenic amine contents (31).

This study stands out as one of the few prospective studies utilizing a crossover design, with continuous diet monitoring conducted by a dietitian to ensure adherence and collect reliable data. The study's limitations include a small sample size and challenges in participant recruitment, particularly exacerbated during the COVID-19 pandemic when adhering to a crossover diet proved demanding. Notably, a few participants withdrew due to their reluctance to revert to the standard mixed diet after experiencing significant symptom alleviation through the histamine-reduced diet. Nevertheless, it is important to highlight that these patients maintained close contact with the study coordinator throughout various diet periods, ensuring meticulous adherence to the diverse diets tested during the study duration.

In conclusion, this prospective study demonstrates that the histamine-reduced diet remains a superior diagnostic tool for patients with suspected non-IgE mediated food-related histamine hypersensitivity, while the analysis of the DAO levels showed to be limited as a diagnostic tool. Changes in DAO do not seem to be related to type of diet and this was consistent in both patients and controls. In this context, larger prospective studies with more motivated participants are necessary to further explore the potential utility of measuring DAO levels in this specific patient population.

**Declarations**

Data Availability Statement: The data that supports the findings of this study are available upon reasonable request from the corresponding author (JvO). The data are not publicly available due to local legislation related to data derived from patients’ medical records.

**Acknowledgments**

Author Contribution Statement: Jenny van Odijk (JvO), Georgios Rentzos (GR) and Linda Ekerljung (LE) designed the study. JvO and Adina Weisheit (AW) collected the data. JVO and GRS analysed the data. All authors contributed to the manuscript and have read and agreed to the submitted version.
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Ethical Approval: The study was approved from the ethics review authority in Gothenburg, Sweden (Document no. T351-17).

Competing Interests: None of the authors declare no competing interests.

References

1. EFSA. Scientific opinion on risk based control of biogenic amine formation in fermented foods.; 2011.


Figures

Figure 1

The study design. Patient and control group allocation was randomized for two discrete 3-week dietary interventions: a low histamine diet (LHD) and an ordinary mixed diet (MXD). Blood samples for the quantification of diamine oxidase (DAO) were obtained at three distinct time points: firstly, prior to the initiation of dietary intervention (designated as DAO1); secondly, following three weeks of dietary adherence to the MXD; and lastly, subsequent to three weeks of dietary exchange, wherein patients
transitioned between LHD and MXD. Throughout the entirety of this six-week dietary intervention period, detailed symptom records were meticulously maintained by the patients within designated symptom diaries.

**Patients:**

![Box plot for patients showing DAO baseline, DAO after HRD, and DAO after OMD.]

**Controls:**

![Box plot for controls showing DAO baseline, DAO after HRD, and DAO after OMD.]

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**Figure 2**
Box plots of logarithmic serum values of DAO in patients and healthy controls using the Friedman’s ANOVA test.