Diamine oxidase determination in serum

Low assay reproducibility and misclassification of healthy subjects

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Bestimmung der Diaminoxidase im Serum – geringe Reproduzierbarkeit der Bestimmung und Fehlklassifizierung von gesunden Personen

Keywords
Histamine – Diamine oxidase – Diagnostic test – Histamine-induced symptoms

Summary

Background: Impaired histamine degradation based on reduced diamine oxidase (DAO) activity has been suggested to cause symptoms mimicking an allergic reaction.

Aim: To test whether patients presenting with possible histamine-induced symptoms have a low serum activity of DAO compared to healthy subjects.

Methods: Sera from 11 patients with a clinical history of potentially histamine-induced symptoms were included together with sera from 18 healthy subjects and 2 pregnant women having no such symptoms. The DAO activity in serum was quantified using a commercial enzyme immunoassay in three repeated measurements. A subset of sera was furthermore analyzed at a service laboratory.

Results: Coefficients of variation between the three measurements of the individual sera were 5–138 %, with a mean of 35 %. The average deviation between the measured and the given value for a control sample included in the test kit was 86 %. Only 11 out of the 31 subjects were uniformly classified in all three runs. Among the healthy subjects, 9–12 out of 18 showed reduced or highly reduced activities; in the patient group, 0–5 out of 11 showed reduced or highly reduced activities in the three measurements. No significant differences were found between the healthy subjects and the patient group in two measurements, but one experiment surprisingly demonstrated a significantly lower mean level of activity, measured by histamine-degrading units (HDU/ml), for the healthy subjects. A subset of the sera were sent to a commercial service laboratory, and here too misclassification occurred. This could be due to reproducibility problems, since the same sample was divided into six aliquots and allocated into different classes in two of six cases.

Conclusion: The high interassay variation and perhaps an incorrectly defined normal range caused misclassification in more than half of the cases. Using a commercial immunoassay, it was not possible to distinguish healthy subjects from patients showing potentially histamine-induced symptoms.

Schlüsselwörter
Histamin – Diaminoxidase – Diagnoseverfahren – histamininduzierte Symptome

Zusammenfassung

Hintergrund: Ein aufgrund einer zu geringen Diaminoxidaseaktivität (Diaminoxidase, DAO) verringerter Histaminabbau steht im Verdacht, Symptome zu verursachen, die einer allergischen Reaktion ähneln.

Ziel: Ziel der Arbeit war es, zu prüfen, ob Patienten mit potenziell histamininduzierten Symptomen – verglichen mit gesunden Probanden – eine niedrigere DAO-Aktivität im Serum haben.

Methoden: Analysiert wurden die Seren von 11 Patienten mit möglicherweise histamininduzierten Symptomen sowie die Seren von 18 gesunden Probanden und zwei schwangeren Frauen ohne Symptome. Die DAO-Aktivität wurde mit Hilfe eines handelsüblichen Enzym-Immunassays gemessen, die Messungen wurden dreimal wiederholt. Ein Teil der Seren wurde zudem in einem externen medizinisch-diagnostischen Labor analysiert.

Ergebnisse: Die Variationskoeffizienten bei den drei Messungen der einzelnen Seren lagen zwischen 5 und 138 % – mit einem Mittel von 35 %. Die mittlere Abweichung zwischen den gemessenen Werten und den Werten eines Kontrollbeispiels, das im Testkit eingeschlossen war, betrug 86 %. Nur elf der insgesamt 31 Personen konnten in allen drei Messungen eindeutig klassifiziert werden. 9–12 der 18 gesunden Probanden zeigten reduzierte oder stark reduzierte Aktivitäten, 0–5 der 11 Patienten zeigten reduzierte oder stark reduzierte Aktivitäten bei al-

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len drei Messungen. Bei zwei Messungen gab es keine signifikanten Unterschiede zwischen den gesunden Probanden und der Patientengruppe, aber die dritte Messung zeigte überraschend ein signifikant niedrigeres mittleres Aktivitätslevel für die gesunden Testpersonen – gemessen anhand von Histaminabbau-Einheiten (HDU/ml). Ein Teil der Seren wurde an ein kommerzielles Service-Laboratorium geschickt. Hier traten ebenfalls Fehlklassifizierungen auf. Dies könnte an Problemen mit der Reproduzierbarkeit liegen, denn dieselbe Probe wurde in sechs Aliquote geteilt und in zwei von sechs Fällen zwei verschiedenen Klassen zugewiesen.

Zusammenfassung: Für die Fehlklassifizierungen in mehr als der Hälfte der Fälle sind die hohen Schwankungen zwischen den Messungen und vielleicht ein nicht korrekt definierter Normalbereich verantwortlich. Bei der Verwendung eines handelsüblichen Immunassays war es nicht möglich, gesunde Personen von Patienten mit potenziell histamininduzierten Symptomen zu unterscheiden.

Introduction

Histamine is a biogenic amine derived from the decarboxylation of the amino acid histidine, a reaction catalyzed by the enzyme L-histidine decarboxylase. It is involved in immune responses (e. g., in allergy where it is produced and released by mast cells and basophils) as well as in regulating physiological functions in the gut; it also acts as a neurotransmitter. Histamine can cause undesirable effects if consumed in excess (scombroid poisoning) [1], and it has been suggested that symptoms may also occur as a result of inadequate histamine catabolism.

Biological inactivation of histamine occurs via oxidative deamination, catalyzed by diamine oxidase (DAO) [2]. The major anatomical sites of DAO expression are the placenta, kidney, and intestine [3]. DAO catabolism of histamine in the intestine reduces the uptake of ingested histamine. This helps to control the histamine concentration locally and in the blood. Normally, the human DAO plasma level ranges from 15 to 50 U/ml, but during pregnancy the DAO level is elevated by up to 500-fold [4].

Histamine occurs in food as a result of microbial enzymes converting histidine to histamine. All foods subjected to microbial fermentation in the manufacturing process contain histamine. Included in this category are aged cheeses, cured meat, fermented foods (e. g., sauerkraut), and alcoholic beverages.

It has been suggested that reduced levels of DAO in plasma may lead to histamine-induced symptoms upon ingestion of histamine-rich food [5, 6]. This has led to the development of commercially available assays and service laboratories that measure the DAO level in serum, but data on the validation and relevance of such measurements are sparse.

The aim of this study was to test the reproducibility of DAO serum measurements and to compare the results from such measurements in healthy subjects and in patients with potential histamine-induced symptoms compatible with reduced histamine catabolism.

Materials and methods

Subjects

As a part of the diagnostic process, we analyzed sera from 11 patients (8 female, age 29–60 years;, 3 male, age 36–48 years) with clinical symptoms potentially caused by histamine intolerance (symptoms such as angioedema, joint pains, fatigue, hypotonia, headache, nausea, burning sensation in the mouth, itching, flushing, meteorism, diarrhea, and stomach ache). None of the patients had IgE-mediated food allergy. In addition, sera from 18 healthy subjects (16 females) and 2 pregnant women, all with no history of food allergy or signs of symptoms indicating histamine intolerance (age 23–62 years), were also included.

Quantitative determination of DAO in serum using D-HIT

Blood samples were collected, centrifuged for 10 min at 2,000 g, and sera were stored at –20°C until further use. We used an enzyme immunoassay for the quantitative determination of histamine degradation activity by DAO in serum (D-HIT kit, SCIOTEC Diagnostic Technologies Vienna, Austria). The assay was performed according to the manufacturer's instructions. In brief, serum was incubated overnight (18–26°C) in a 96-well plate containing histamine. During this time the DAO present in serum would degrade histamine. The remaining histamine was then acetylated and measured in a competitive ELISA using a 6-point standard curve. The results are given as histamine-degrading units (HDU) per milliliter.

Quantitative determination of DAO activity in serum by a service laboratory

A subset of the sera were measured at a commercial service laboratory (GANZIMMUN.de) where a radioimmunoassay (RIA) was used for the quantitative (U/ml) determination of DAO activity in serum.

Results

Classification of serum samples according to DAO activity using a commercial assay

We tested 31 samples of sera (2 from pregnant women, 18 from healthy subjects, and 11 from patients) for DAO activity in three separate experiments using three batches of the D-HIT kit.

As presented in Table 1 (part a), the coefficients of variation (CV) % ranged from 5 to 138 % between the individual sera with a mean of 35 %, which is above the 20 % given by the manufacturer for interassay variation (the intra-assay variation was 9 % between samples, which is in accordance with the stated intra-assay variation of less than 15 %). Furthermore, the average deviation between the measured and the given value for the assay control sample included in the kit was 86 %, with the measured value always higher than the value reported on the vial (data not shown).

Among the healthy subjects, 9–12 out of 18 showed reduced or highly reduced DAO activity, while in the patient group, 0–5 out of 11 showed reduced or highly reduced activity.

Interestingly, in experiment 1 and 3 no significant difference in DAO activity was found between the healthy subjects (74 ± 34 and 91 ± 71) and the patients (85 ± 30 and 102 ± 57), whereas experiment 2 surprisingly demonstrated a significantly lower mean HDU/ml level among the healthy subjects (79 ± 27 vs. 114 ± 29, p = 0.0037, two-tailed t test). When the means of three measurements (81 ± 41 vs. 101 ± 31) were used for comparison, no statistical differences were found between the groups. Owing to the lack of an independent confirmatory diagnostic criterion for histamine-induced symptoms in the patients, we can not document that the patients had a reduced level of DAO in serum, but one would expect at least the healthy subjects to be classified as normal.

In all three experiments, the sera from pregnant women were classified as normal and the results were, as expected, considerably higher compared to the controls and patients, but the CV % was still above the level claimed by the manufacturer.

Table 1

Classification of sera according to DAO activity
(a: The same batch of serum was analyzed in three independent experiments. The mean and CV percentage are given.
b: Results from the service laboratory. Only a subset of the sera were analyzed)

a						b
	Experiment 1	Experiment 2	Experiment 3	mean	CV	Service Lab
	HDU	HDU	HDU	HDU		U/ml
Preg 1	230	157	324	237	35 %	> 80
Preg 2	212	184	375	257	40 %	> 80
C1	45	66	57	56	18 %	9.8
C2	71	48	88	69	29 %	17.1
C3	96	88	97	94	5 %	
C4	**35**	65	**36**	46	38 %	10.7
C5	69	115	131	105	31 %	29.2
C6	106	67	144	105	36 %	18.2
C7	143	154	284	194	41 %	
C8	41	69	42	50	32 %	
C9	92	78	80	84	9 %	15,7
C10	**3**	**38**	**3**	**15**	138 %	11.1
C11	41	72	**25**	46	52 %	
C12	133	87	218	145	45 %	22.4
C13	74	72	102	82	21 %	13.4
C14	75	91	69	78	15 %	7.5
C15	77	104	92	91	14 %	21.6
C16	55	69	**32**	52	36 %	
C17	100	51	**11**	54	82 %	
C18	74	95	125	98	26 %	21.3
P1	74	85	81	80	7 %	**1.4**
P2	142	157	248	182	31 %	3.7
P3	86	131	139	118	24 %	18.1
P4	65	100	116	94	28 %	18.3
P5	71	96	98	88	17 %	3.3
P6	109	102	125	112	11 %	16.4
P7	121	93	74	96	25%	6.2
P8	82	103	78	88	15%	10.5
P9	64	85	93	81	19%	
P10	Nd	180	49	114	81%	**< 0.3**
P11	**35**	122	**17**	58	97%	

D-Hit kit classification (a)	*Service laboratory classification (b)*
Normal DAO: > 80 HDU	*> 10 U/ml Histamine intolerance not likely*
Reduced DAO: 40–80 HDU	*3–10 U/ml Possible histamine intolerance*
Highly reduced DAO: < 40 HDU	***0–3 U/ml Histamine intolerant***

DAO, diamine oxidase; HDU, histamine-degrading units; Nd, not determined; Preg, pregnant subject; C, control subject; P, patient; CV, coefficient of variation

Serum DAO activity determined by service laboratory

The DAO activity was measured in a subset of the sera at a commercial service laboratory. As shown in **Table 1** (part b), 2 out of 12 healthy subjects were classified as having possible histamine intolerance (> 10 U/ml). Surprisingly, healthy subject no. 10 was classified as being healthy, albeit having the lowest DAO activity measured with the D-HIT kit. Among the patients 5 out of 9 were found to be "possible histamine-intolerant" or "histamine-intolerant," whereas the pregnant women were both classified as "histamine intolerance not likely," with a DAO level above the detection limit. The different distributions seen between the classifications might reflect the reproducibility of the testing. Therefore, we had one serum sample from a healthy subject divided into six aliquots, where two aliquots were shipped and analyzed at the same time (three rounds in total). As seen in **Table 2**, the results from two of the six analyses differed from the other four.

Discussion

Our small study of DAO activity determination revealed a higher assay-to-assay variation than suggested by the manufacturer. The large variation (in CV %) is probably the reason why less than half of the subjects were classified uniformly (see Table 1, part a) when repeating the testing three times. An obvious explanation for the poor reproducibility of the ELISA when conducted in our laboratory is sheer technological incompetence on our part. However, the average intra-assay variations on duplicate determinations speak against this, being only 9 % and thus within the 15 % limit suggested by the manufacturer. Since only a single sample from each person was tested, biological variation (which is reported to be small [7]) could be eliminated as the reason for variability. The possibility that DAO is broken down or rendered inactive during the repeated freezing and thawing of samples can also be excluded since there was no general decreasing trend from experiment 1 to 3. The fact that the internal control of the assay also showed a marked variation points to the standardization of the assay as a possible cause of the variability. It could be argued that one could use the internal control to adjust the values of the other samples in the set-up, but since both the offset and the slope could be the cause of the variation this would seem to be a procedure prone to introducing more errors.

To eliminate the factor of our own laboratory, we shipped the samples to an external service laboratory. Based on a single sample, the assay-to-assay CV % was indeed reduced to 22 % compared to an average CV % of 35 % that we obtained using the ELISA. However, the assay performed by the service laboratory is based on a different assay principle and standardization, and more samples would have to be tested in order to obtain a more precise estimate of the true reproducibility of this assay. The results provided by the external laboratory emphasize another point, which is the normal ranges. Even though the external laboratory only identified two (17 %) samples from the healthy subjects as „possible histamine intolerant" (in comparison to 44 % as „possible histamine intolerant" and 15 % „histamine intolerant" with the ELISA), it is important for a diagnostic test to have a well-defined normal range that would include 95 % of persons without the alleged disease.

It has been debated whether a low plasma level of the histamine-catabolizing enzyme DAO in combination with ingestion of histamine-rich foods can lead to systemically increased levels of histamine and thus symptoms – so-called histamine intolerance – but this was not the subject of the present study. Without reliable measurements of plasma or serum levels of DAO, it will be difficult to study this hypothesis further.

Table 2

Reproducibility of classification – repeated analysis of serum from a healthy subject

Test round 1	Test round 2	Test round 3
11.2 U/ml	9.3 U/ml	10.1 U/ml
12.4 U/ml	11.2 U/ml	5.6 U/ml

>10 U/ml Histamine intolerance not likely

3–10 U/ml Possible histamine-intolerant

0–3 U/ml Histamine-intolerant

Conclusion

In conclusion, using the commercial D-HIT kit, it was not possible to distinguish healthy subjects from patients with possible histamine-induced symptoms. However, since we had no alternative way of defining a true-positive diagnosis of alleged histamine intolerance, it is difficult to know whether we selected the correct patients. Nevertheless, the high interassay variation and perhaps an incorrectly defined normal range caused misclassification in nearly two thirds of the cases. Therefore, owing to the large analytical variation, we conclude that the D-HIT assay it is not able to reliably identify healthy subjects or those suspected of being histamine-intolerant.

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Conflict of interest

On behalf of all authors, the corresponding author states the following: Per S. Skov is scientific advisor at RefLab ApS. Heidi J. Schnoor, Holger Mosbech, Lars K. Poulsen, and Bettina M. Jensen do not have any conflict of interests.

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