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Elevated Postmortem Tryptase in the Absence of Anaphylaxis

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ABSTRACT: Elevations in tryptase, a recently discovered mast cell enzyme, have been proposed as a postmortem indicator of fatal anaphylaxis. The previous studies had limited numbers of controls and thus the specificity of the test with postmortem samples was not known. Therefore, tryptase was evaluated in postmortem blood samples from 49 autopsy cases where there was no evidence of fatal anaphylaxis. The tryptase was above the normal serum threshold of 1 nanogram/mL (ng/mL) in 31 of these cases. Twenty-four cases had values in the 1 to 5 ng/mL range, two cases were between 5 and 10 ng/mL, and five were greater than 10 ng/mL. One autopsy specimen had a tryptase value of 106 ng/mL. The postmortem interval and the specimen storage condition did not appear to correlate with these elevations in tryptase. Although elevations in the postmortem tryptase remain an important supporting finding in the diagnosis of fatal anaphylaxis, it should not be used alone as the sole criterion for the postmortem diagnosis of anaphylaxis.

KEYWORDS: pathology and biology, anaphylaxis, tryptase, death, postmortem

The postmortem diagnosis of fatal anaphylaxis is complicated by the relatively nonspecific pathologic findings seen in these deaths. Occasionally, angioedema of the larynx and accompanying pulmonary emphysema are seen along with the perhaps more common nonspecific findings of visceral congestion and pulmonary edema [*1,2*]. Often, no significant pathologic findings are discovered at autopsy.

Activation of mast cells is triggered by the interaction of antigen with membrane bound IgE. This interaction leads to the release of a variety of chemical mediators that are an integral part of the anaphylactic process [*3–5*]. Histamine is an important marker of mast cell activation, but it is unsuitable for the postmortem diagnosis of anaphylaxis because it has a very short half life in the circulation and it can also be released from postmortem basophils in the blood sample in the absence of anaphylaxis [*7*]. The neutral protease tryptase has a longer serum half-life and it is found almost exclusively in tissue mast cells, which makes it a specific marker for systemic mast cell events [*6–8*]. Recently, Yunginger et al. [*9*] and Ansari et al. [*10*] have published reports demonstrating elevated tryptase levels in the postmortem sera of patients who died from anaphylaxis.

Our interest in the use of tryptase in postmortem evaluation of anaphylaxis followed an investigation of the death of an obese, 49-year-old woman who was found dead in her bed unexpectedly. She had a nonspecific mild psychiatric history for which she was taking thioridazine. She also had a long history of shortness of breath. Two days prior to death she had been started on Omniflox (temafloxacin) to treat a urinary tract infection. The autopsy revealed a moderate dilated cardiomyopathy associated with a congestive hepatosplenomegaly and early ischemic hepatic necrosis. The postmortem blood thioridazine and mesoridazine were 6.5 μg/mL and 3.9 μg/mL, respectively. We felt that the cardiomyopathy and the elevated thioridazine levels, in combination, were potential causes of death in this case [*11*]. However, at the time of this death, Omniflox had just been removed from the market because of reports of anaphylacticlike reactions, some of which were fatal [*12,13*]. Therefore, the blood was subsequently assayed for tryptase and a value of 143 ng/mL was measured. A test for Omniflox specific IgE was not available. Although the elevated tryptase level was strongly suggestive of a systemic mast cell event (such as anaphylaxis), the clinical history from others living in the house with the decedent provided no evidence supporting anaphylaxis as the cause of death. We were concerned about the specificity of tryptase alone to diagnose anaphylaxis and noted that the study of Yunginger et al. [*9*] and Ansari et al. [*10*] included postmortem sera from 6 and 1 controls, respectively. Therefore, we examined a larger population of routinely collected postmortem samples to assess the diagnostic efficacy of postmortem tryptase testing.

Materials and Methods

Test Samples

A trial blood sample was recovered at autopsy from two series of consecutive autopsies of 27 and 22 bodies, respectively. In none of these cases was anaphylaxis suspected as a cause of death. The postmortem interval prior to specimen collection was recorded as was the cause of death. Two series of deaths were used to test the effect of different postmortem specimen storage conditions. The first series of autopsies were done in South Dakota and the second set in North Carolina. In the first group the whole blood was stored for variable periods of time (up to several days) at 4 C prior to being frozen and stored at −72 C. The second group was processed identically, except the blood sample was immediately frozen at −72 C.

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Portions of these data were presented at the American College of Allergy and Immunology annual meeting in Atlanta, GA, November, 1993.

Tryptase Assay

A solid phase immunoradiometric assay kit manufactured by Kabi-Pharmacia Diagnostics was used to measure tryptase in the blood samples. In this assay the tryptase in the sample reacts with monoclonal anti-tryptase antibody linked to the solid phase and the bound tryptase molecules are subsequently detected with ^{125}I-labelled anti-tryptase antibody. Standards, controls and patient samples were tested in duplicate. The stability of tryptase in a blood serum matrix was investigated by incubating a specimen at three temperatures (−20 C, 4 C, and 23–25 C) for 25 days.

Results

The postmortem tryptase values, cause of death, postmortem interval and age/sex information are shown for the two study populations (Tables 1 and 2). There was no evidence that any of these 49 deaths were the result of an anaphylactic reaction. However, only 18 of the 49 specimens had a tryptase concentration less than 1 ng/mL, the upper limit for 95 to 98% of healthy, normal individuals in the absence of a systemic mast cell event. Table 3 summarizes the tryptase values for the two groups of autopsy samples. These data demonstrate that a significant percentage of routinely procured autopsy specimens contain elevated tryptase values.

Since the sera were not assayed immediately after collection, we examined the stability of serum tryptase over a 25 day interval. The data in Fig. 1 illustrate that the assayed tryptase concentration in a blood serum specimen did not change significantly over a 25 day period at any of the three storage conditions. Similar results were observed with tryptase specimens in the <1 ng/mL range.

Discussion

An ideal postmortem diagnostic marker for anaphylaxis would be a test that had high specificity when performed on actual blood samples that are routinely obtained at autopsy. Obviously, in most death investigations, there will be significant variation from sample to sample with regard to postmortem interval, specimen handling and storage conditions. It was decided to investigate the utility of the test under these routine procurement conditions. We observed elevated tryptase values in a high percentage of specimens collected from persons who died from nonanaphylactic causes. These data demonstrate that an elevated postmortem tryptase obtained at autopsy cannot be used to establish the cause of death as anaphylaxis without additional supporting data. It should be noted that the study by Yunginger et al. [9] also reported elevated tryptase concentrations in specimens collected from persons dying from nonanaphylactic causes. However, their control specimens were obtained from blood and other fluids both before and after death and in some cases the serum was immediately prepared from the blood specimens and frozen. The individual data were not reported and thus it is not clear which specimens had elevated tryptase values. Once the specimen has been obtained, the storage conditions do not appear to be important. The specimens in Table 2 were all immediately frozen at −72 C and these showed elevations similar to those in Table 1. Further, the data in Fig. 1 also demonstrate the stability of this enzyme when stored at various temperatures. Nevertheless, it is possible that rigidly standardized specimen collection protocols might improve the specificity of the test in postmortem cases. However, this would obviously limit the usefulness of the test in most death investigations.

TABLE 1—*Postmortem tryptase levels and autopsy findings for the first study population.*

Case #	Cause of Death	Age	Sex	PMI[a]	Tryptase Concentration (ng/mL)
1	Multiple trauma	19	M	12	<1
2	COPD[b]	69	M	20	<1
3	CO poisoning	94	M	4	<1
4	ASCVD[c]	65	M	4	<1
5	Multiple trauma	2	F	8	<1
6	ASCVD	84	F	4	<1
7	Amitriptyline overdose	31	F	2	<1
8	COPD	94	M	20	<1
9	Pneumonia	76	M	12	<1
10	Amitriptyline overdose	35	M	2	<1
11	CO poisoning	18	F	4	<1
12	Drowning	1	M	14	<1
13	ASCVD	68	M	4	<1
14	Undetermined	47	M	16	1.1
15	Chronic alcohol abuse	43	M	3	1.1
16	CO poisoning	9	F	8–12	1.3
17	CO poisoning	12	M	8–12	1.6
18	Aortic aneurysm	54	M	3	1.6
19	Gunshot wound	52	M	2	1.7
20	Seizure disorder	28	F	12	2.1
21	CO poisoning	21	M	24	2.1
22	ASCVD	42	M	18	4.0
23	ASCVD	62	F	8	4.7
24	Pulmonary embolism	57	F	2	5.1
25	Salicylate overdose	18	F	24	23.1
26	Multiple trauma	26	M	48	24.0
27	Multiple trauma	65	F	4	106.

[a]PMI, postmortem interval in hours.
[b]COPD, chronic obstructive pulmonary disease.
[c]ASCVD, atherosclerotic coronary vascular disease.

TABLE 2—*Postmortem tryptase levels and autopsy findings for the second study population.*

Case #	Cause of Death	Age	Sex	PMI[a]	Tryptase Concentration (ng/mL)
28	ASCVD[b]	53	M	12	<1
29	ASCVD	36	M	22	<1
30	Chronic alcohol abuse	43	M	23	<1
31	Head trauma	29	M	19	<1
32	Cocaine overdose	35	M	9	1.0
33	Theophylline overdose	46	F	32–38	1.1
34	SAH[c]	54	M	18	1.1
35	ASCVD	49	M	28–40	1.5
36	ASCVD	44	M	20	1.5
37	Head trauma	58	M	5–8	1.6
38	Aortic vascular disease	79	F	9	2.0
39	Aortic vascular disease	42	M	9–12	2.2
40	SLE[d]	34	F	20–29	2.3
41	Gunshot wound	21	M	10	2.4
42	Multiple trauma	23	M	11	2.9
43	Pulmonary emboli	47	M	10	3.1
44	Salicylate overdose	39	M	13	3.5
45	Chronic alcohol abuse	46	F	22–24	3.7
46	ASCVD	46	M	18	4.9
47	Gunshot wound	36	M	14	5.4
48	Gunshot wound	30	M	10	20.1
49	ASCVD	38	F	2	32.9

[a]PMI, postmortem interval in hours.
[b]ASCVD, atherosclerotic coronary vascular disease.
[c]SAH, subarachnoid hemorrhage.
[d]SLE, systemic lupus erythematosus.

TABLE 3—*Distribution of tryptase values in the 49 autopsy cases.*

Tryptase (ng/mL)	Number (%)
<1	18 (37%)
1.1–2.0	12 (24%)
2.1–5.0	12 (24%)
5.1–10.0	2 (4%)
>10.0	5 (10%)

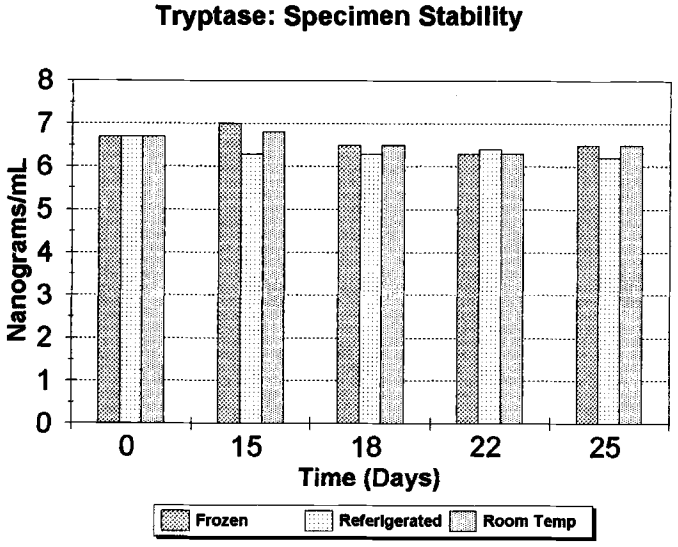

FIG. 1—*Tryptase: specimen stability. The specimen was assayed on day zero and then stored either frozen (−20 C), refrigerated (4–6 C) or at room temperature (23–25 C) prior to reassay.*

Although an elevated postmortem tryptase alone did not appear to be specific for anaphylaxis, it might have better specificity if a higher threshold (for example, 10 ng/mL) were used. However, since a few specimens from the set of autopsy cases were also found to be significantly above this value, the specificity might not be greater than 90% (see Table 3). Nevertheless, the tryptase test remains a useful test in evaluating unexplained deaths, when other clinical information suggest an anaphylactic reaction. Thus, in autopsy cases with an appropriate clinical history and the finding of allergen-specific IgE, the elevated tryptase represents important supporting evidence upon which to base a diagnosis of anaphylaxis [*14,15*].

In contrast to the postmortem evaluation, our experience with the tryptase assay is that the test has high specificity for anaphylaxis if the specimen is obtained while the patient is still living. False positives appear to be rare and greater than 95 to 98% of normal healthy individuals have values less than 1 ng/mL. Additional studies are needed to determine the reason for the apparent lack of specificity in postmortem vs antemortem evaluations.

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