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Reliable Postmortem Molecular Diagnosis of Anaphylaxis Co-localization of Mast Cell Degranulation and Immunoglobulin E in Allergic Throat Tissues

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Abstract: Anaphylaxis is a serious reaction that may cause death in half an hour without diagnostic characteristic in autopsies. Mast cell (MC) degranulation combined with immunoglobulin E (IgE) plays the key roles in anaphylaxis. Unavailability of serum and instability of measured serum in postmortem diagnoses sometimes limit the opinion of medical experts. Allergic tissues are more accessible than serum, and there is a little research on degranulated mast cells and IgE in different human tissues, whereas we hardly know whether the expression will keep stable over the increasing postmortem interval (PMI). In this research, we examined the mast cell counts and degranulation rates and IgE contents in human throat, lung, and intestine tissues and preliminarily investigated the correlation of these markers with PMI in anaphylaxis-associated death. Allergic samples showed a significant increase in mast cell degranulation accompanied by an increase in IgE levels than the control group, but the expression was not significantly correlated with increasing PMI only in throat tissues. Elevated mast cell degranulation combined with increased IgE levels may be a reliable biomarker for forensic diagnosis of human tissues due to IgE-mediated allergic sudden death.

Key Words: mast cell degranulation, IgE, postmortem interval, anaphylaxis

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KEY POINTS

1. To seek a convenient and practical path, morphological targets for anaphylaxis-associated death are a key problem, which is the main purpose of this article.
2. Our test method is convenient and inexpensive, which could be used in postmortem diagnoses.
3. It is the first time to study the relationship between mast cell degranulation and IgE and the postmortem interval (PMI) in human allergic tissue.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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4. Our study found that IgE positivity was higher in allergic human tissues and inversely related to PMI in the intestine (not related to PMI in the throat and lung).
5. We have first proved that the mast cell degranulation expressed highly in the allergic human tissues and significantly positively correlated with the PMI in the lung.
6. We demonstrate that mast cell degranulation combined with the increased level of IgE in throat tissue would be a reliable biomarker in the forensic diagnosis of decomposed bodies for IgE-mediated allergic sudden death.

Anaphylaxis is a severe systemic reaction that is mainly caused by the immunoglobulin E (IgE)-mediated release of mediators from basophils and mast cells.¹ The National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network define anaphylaxis as a serious reaction with rapid onset that may cause death in half an hour, without diagnostic characteristic other than organ congestion and edema in autopsies.² Forensic diagnosis is often based on the exclusion of other possible causes, such as asphyxia, intoxication, drugs, traumatic death, and other fatal diseases, in combination with a history of allergy.

Recently, with its apparently increasing incidence, the lack of a convenient and reliable biomarker for forensic identification of anaphylaxis has received increasing attention.³ The key problem is to find a convenient and practical target. Increased levels of serum tryptase, IgE, histamine, platelet-activating factor, and eosinophil cationic protein have been studied for use as biomarkers of anaphylaxis-associated death.^{4–7} However, the opinion of medical experts is limited by the unavailability of serum and the instability of measured serum, because high-quality serum is often unaccessible in forensic medicine. Studies on postmortem stability of these analytes in serum are lacking, except for tryptase, which has a longer half-life and has thus been the focus of research. Yunginger et al⁸ demonstrated early in 1991 that elevated levels of serum mast cell tryptase and IgE can be indicators of anaphylactic events in postmortem examination. Research has shown the usefulness of increased serum tryptase levels in diagnosis of fatal anaphylaxis.^{5,7,9,10} Frustratingly, serum tryptase levels will notably decline with the increasing postmortem interval (PMI).¹¹ The serum tryptase level increases and peaks hours after anaphylactic death. Biochemical or immunological tests may be more feasible when tissues rather than blood are adopted. Recently, several studies suggest that mast cell tryptase can be significantly upregulated in allergic tissues. Such upregulation may occur in other diseases besides anaphylaxis,¹² so it is unsafe to diagnose anaphylaxis from an elevated tryptase level alone. Moreover, IgE, a key factor in allergic reactions and value of which in anaphylaxis diagnosis has been questioned because of its short half-life in serum,¹³ has rarely been studied in human tissues.

In this study, the mast cell (MC) count and degranulation rate and the IgE content in human bodies (throat, lung, and intestine tissues) were first jointly detected by immunohistochemical (IHC) staining. Then dual immunity group dyeing and double immunofluorescent

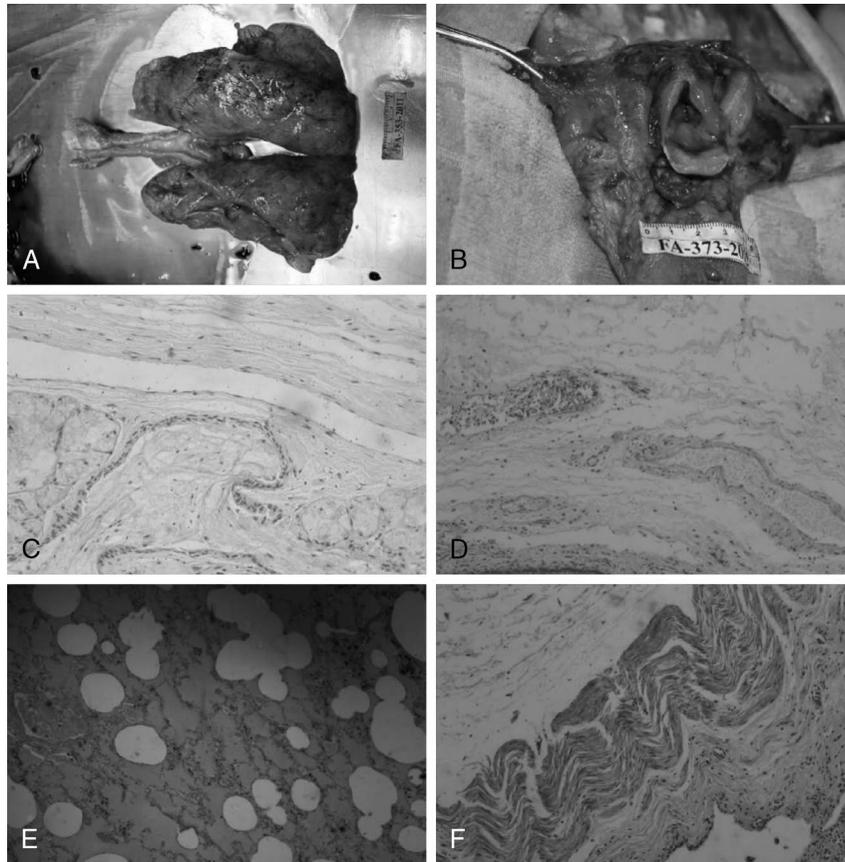


FIGURE 1. A, Allergic lung tissue edema and a narrowed glottis fissure. B, Edema of allergic larynx and throat tissue. The upper left corner of (C) glandular secretion of allergic larynx tissue (HE ×200). D, Congestion and edema of allergic larynx tissue (HE ×200). E, Congestion and edema of allergic lung tissue (HE ×200). F, Muscle spasm of allergic intestinal tissue (HE ×200).

(IF) staining were performed to visually observe the co-localization of mast cells and IgE. Finally, the PMI of the cases ranged from days to weeks, which allowed us to preliminarily investigate the correlation of marker expression in tissues with the time of death in anaphylaxis-associated deaths. It is the first time to study whether the mast cell degranulation and IgE expression will change over the PMI in human allergic tissues. The test method is convenient and inexpensive and can be used in postmortem diagnoses.

MATERIAL AND METHODS

Sample Collection

We obtained throat, lung, and intestine tissues of 72 entire bodies examined by the Judicial Critical Center of Shantou University Medical College, whereas the postmortem results included 20

cases of drug-related anaphylactic sudden death and 52 cases of nonallergic death. The 52 control cases consisted of trauma and heart disease (n = 12 each), cyanide poisoning (n = 6), respiratory infection after multiple organ failure (n = 5), sudden infant death syndrome (n = 4), cerebral hemorrhage and thrombosis (n = 3 each), heat exhaustion and postpartum hemorrhage (n = 2 each), and suicide, electric shock, and septicemia (n = 1 each).

The mean age of cases was 34.1 years (0–75 years, 46 males), and the average PMI was 12.7 days (0–85 days). All tissues were handled with strict adherence with Chinese law and were approved by the ethics review boards in China.

Immunohistochemical Staining

All specimens were fixed in 4% neutral buffered formalin for 12 hours, then embedded in paraffin, and cut into consecutive

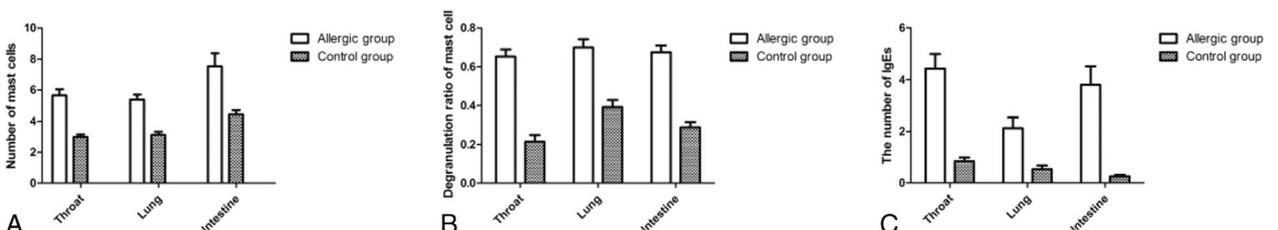


FIGURE 2. A, Numbers of mast cells, (B) degranulation ratio of mast cells, and (C) number of IgE-positive cells between allergic group and control group in the throat, lung, and intestinal tissues.

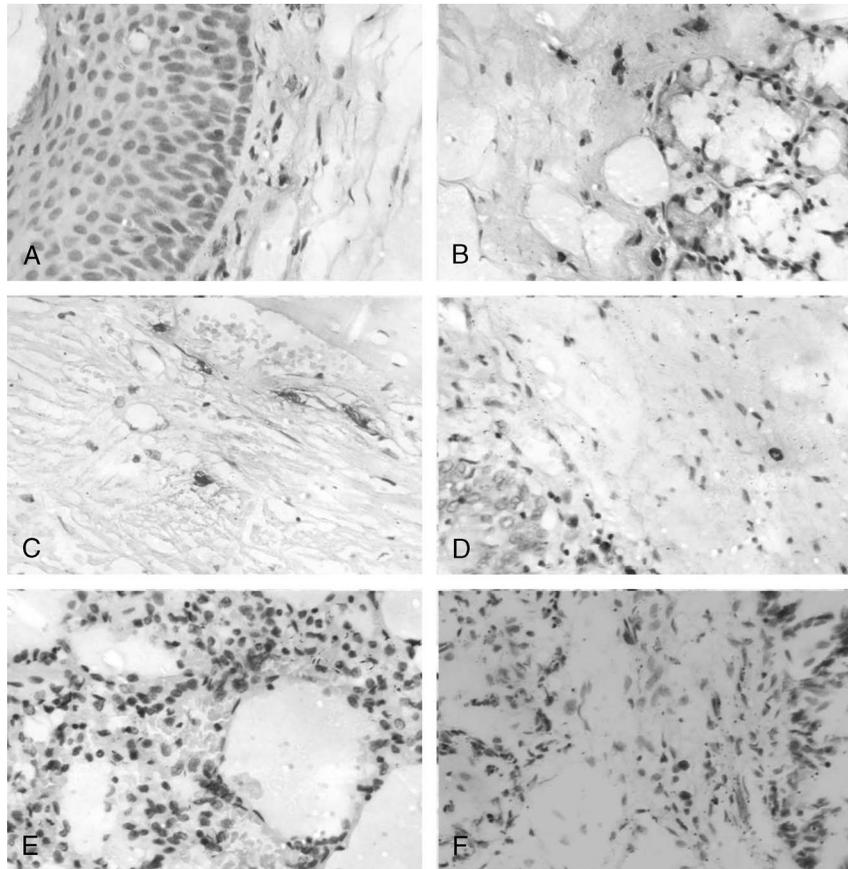


FIGURE 3. A–C, Much expression and degranulation of MC in allergic larynx tissue (IHC $\times 400$). D, Poor expression of MC in nonallergic larynx tissue (IHC $\times 400$). E, MC expression in allergic lung tissue (IHC $\times 400$). F, MC poor expression in nonallergic lung tissue (IHC $\times 400$). MC; mast cells.

sections (4 μm thick). Then, the sections were baked at 60°C for 3 hours, deparaffinized with xylene, and rehydrated. Before bright field microscopy, a group of sections were stained with hematoxylin and eosin (HE) for morphological observation. Replicate sections were treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity and were then incubated with goat serum to block nonspecific binding. After heating in a pressure cooker (for 3 minutes) in 0.01 M citrate buffer (pH 6.0), the sections were incubated with primary antibodies for mouse antihuman mast cell tryptase (1:200, M-0374; Shanghai Long Island Biotech, China) and rabbit antihuman IgE (1:200, GTX73360; Gene Tex) overnight at 4°C. After washing with phosphate buffer solution, the sections were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody for 30 minutes before visualization with 3-amino-9-ethylcarbazol-AEC and counterstaining with hematoxylin. The positive mast cells stained brown in the cytoplasm, and IgE-positive cells stained brown in the cytoplasm or on the membrane. We photographed 10 randomly selected fields in the immunohistochemically stained samples at 400X magnification under a power microscope (Leica DMRXA2) with an image acquisition system (Leica IM50). Then, the number of positive mast cells or IgE-positive cells in every field was counted, and the final result of each case was determined by calculating the average number of positive cells in the 10 fields. The mean degranulation rate of mast cells was calculated as count of degranulated mast cells in 10 fields/total count of mast cells in 10 fields $\times 100\%$. Mast cell degranulation was defined as mast cells with scattered granules or nuclei deformation.

Double IHC and IF Staining

Double IHC was performed to identify the co-localization of mast cells and IgE. After the mast cells and IgE staining were photographed, sections were placed first in 60°C water to fade the staining and then on a shaker in 80% alcohol for 1 hour. After 3 washes with phosphate buffer solution and incubation in Tris-HCL (pH 1.5) overnight at 4°C, the IHC steps were repeated, and the samples were stained with 3,3'-diaminobenzidine (DAB). The sections were dehydrated and mounted on slides. For double IF staining, duplicate sections were deparaffinized with xylene, rehydrated, and repaired with appropriate methods (as described). Then, the sections were incubated first with a mixture of antibodies for mast-cell tryptase and rabbit antihuman IgE overnight at 4°C and then with a mixture of IF Cy3-labeled goat antimouse IgG (H + I, 1:200, A0521; Beyotime, China) and fluorescein 5-isothiocyanate (FITC)-labeled goat antirabbit IgG (H + I) antibodies (1:200, A0562; Beyotime) for 30 minutes at room temperature before examination under a fluorescence microscope. Images were acquired using a Leica IM50 picture-collection system, and the number of mast cells, amount of mast-cell degranulation, IgE content, and co-localization of mast cells with IgE were analyzed using Image-Pro Plus 6.0.

Statistical Analysis

The data were analyzed using IBM SPSS 21.0 (SPSS Inc, Chicago, Ill). Continuous variables are reported as the mean \pm SD, and the exact 95% confidence interval (CI) is indicated. Unpaired

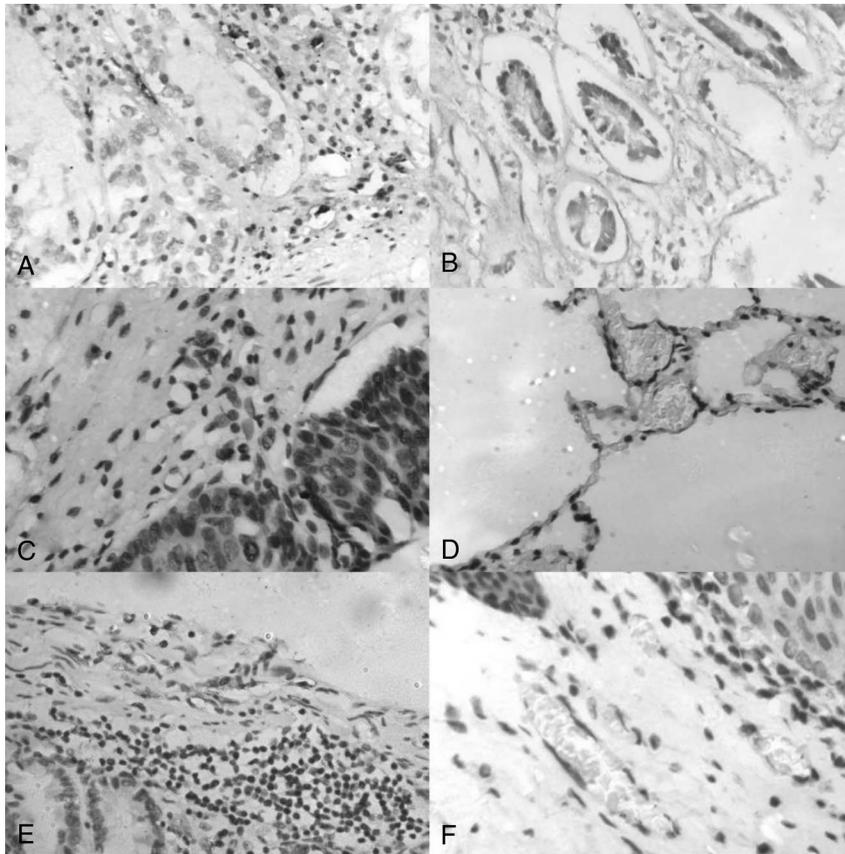


FIGURE 4. A, MC expression in lamina propria of intestinal mucosa in allergic intestine tissue (IHC × 400). B, MC poor expression in nonallergic intestine tissue (IHC × 400). C, Immunoglobulin E expression in allergic laryngeal tissue (IHC × 400). D, Immunoglobulin E expressed in allergic lung tissue. E, Immunoglobulin E expressed in allergic intestine tissue (IHC × 400). F, The negative expression of IgE in nonallergic tissue (IHC × 400).

2-sided Student *t* tests, Kruskal–Wallis one-way analysis of variance, and nonparametric Kruskal–Wallis test were used to compare several means. Spearman rank correlation test was used to analyze the correlation between marker contents and the PMI. *P* < 0.05 was considered statistically significant.

RESULTS

Expression of MC

In the allergic group, the autopsies grossly revealed severe larynx edema, and some cases had a narrowed glottis fissure. The microscopic indications of anaphylaxis included significant congestion of all the organs and nonspecific changes. The activities of the throat cement glands, the congestion of the tissues, and

spams of the intestinal muscle were observed in the allergic tissue; in addition, in the lung, the alveolar walls were enlarged, and the alveolar cavities were filled with pink edematous fluid (Fig. 1). The allergic samples compared with controls showed significantly higher numbers of mast cells (throat 5.66 ± 0.40 vs 2.99 ± 0.15 , lung 5.38 ± 0.33 vs 3.13 ± 0.19 , intestine 7.54 ± 0.85 vs 4.44 ± 0.27) and significantly larger degranulation rates (throat 0.65 ± 0.037 vs 0.21 ± 0.04 , lung 0.70 ± 0.042 vs 0.39 ± 0.04 , intestine 0.68 ± 0.035 vs 0.29 ± 0.03 ; all *P* < 0.01). The MC positive cells were mainly distributed in the laryngeal lamina propria around small blood vessels and cement glands; in the lung, they were mostly located around blood vessels, and a few were located among the pulmonary epithelial cells; in the intestine, they mainly distributed among the glands of the intestinal mucosa and in the

TABLE 1. The Number of Mast Cell (No/hp*) in Allergic Group and Nonallergic Group of Throat Tissue, Lung Tissue, and Intestinal Tissue

| Mast Cell | N | Throat | P | Lung | P | Intestine | P |
|---------------------|----|-----------------|-------|-----------------|-------|-----------------|-------|
| allergic group | 20 | 5.66 ± 0.40 | 0.000 | 5.38 ± 0.33 | 0.000 | 7.54 ± 0.85 | 0.000 |
| none-allergic Group | 52 | 2.99 ± 0.15 | | 3.13 ± 0.19 | | 4.44 ± 0.27 | |

hp*, high power field.

TABLE 2. The Difference Result of Mast Cell Degranulation Ratio (/hp*) in Allergic and Nonallergic Group of Throat Tissue, Lung Tissue, and Intestinal Tissue Degranulation Ratio

| Degranulation Ratio | N | Throat | | Lung | | Intestine | |
|---------------------|----|-----------------|-------|-----------------|-------|-----------------|-------|
| | | Throat | P | Lung | P | Intestine | P |
| allergic group | 20 | 0.66 ± 0.04 | 0.000 | 0.70 ± 0.04 | 0.000 | 0.68 ± 0.03 | 0.000 |
| none-allergic group | 52 | 0.21 ± 0.02 | | 0.39 ± 0.04 | | 0.29 ± 0.03 | |

hp*, high power field.

TABLE 3. The Number of IgE-Positive Cell (/hp*) in Allergic and Nonallergic Group of Throat Tissue, Lung Tissue, and Intestinal Tissue

| IgE | N (Positive/total) | Throat | P | N (Positive/total) | Lung | P | N (Positive/total) | Intestine | P |
|--------------------|-----------------------|-----------|-------|-----------------------|-----------|-------|-----------------------|-----------|-------|
| | | | | | | | | | |
| allergic group | 19/20 | 4.43±0.57 | 0.000 | 15/20 | 1.73±0.42 | 0.000 | 20(20) | 3.80±0.72 | 0.000 |
| non-allergic group | 27/52 | 0.85±0.14 | | 18/52 | 0.25±0.07 | | 52(29) | 0.54±0.15 | |

hp*, high power field.

connective tissue of the submucosa (Figs. 2A, B, 3A–F, 4A, 2; Tables 1, 2).

Detection of IgE

Immunoglobulin E-positive co-located with mast cells. The numbers of IgE-positive cells were significantly higher in the allergic tissues than in the control tissues (throat: 4.43 ± 0.57 vs 0.85 ± 0.14 ; lung: 1.73 ± 0.42 vs 0.25 ± 0.07 ; intestine: 3.80 ± 0.72 vs 0.54 ± 0.15 ; all $P < 0.01$; Figs. 2C, 4C–F; Table 3). In the allergic group, 100% of the IgE-positive cells in intestinal tissues were in intestinal mucosa and submucosa connective tissues, 98% of the cells in throat tissues were in the superficial lamina propria, lamina propria of laryngeal mucosa and around glands, and 75% of the cells in lung tissues were around small blood vessels.

Co-localization of Mast Cell Degranulation and IgE in Human Tissues

Immunofluorescent staining and double IHC revealed IgE positivity in the cytoplasm and membrane of mast cells with scattered granules (Figs. 5, 6). The mast cells in intestinal tissues of myocardial infarction cases had no scattered granules, and the IgE expression was negative. In contrast, in the cases of electric shock death, the mast cell number increased with degranulation around blood vessels, and the IgE expression was negative (Fig. 7).

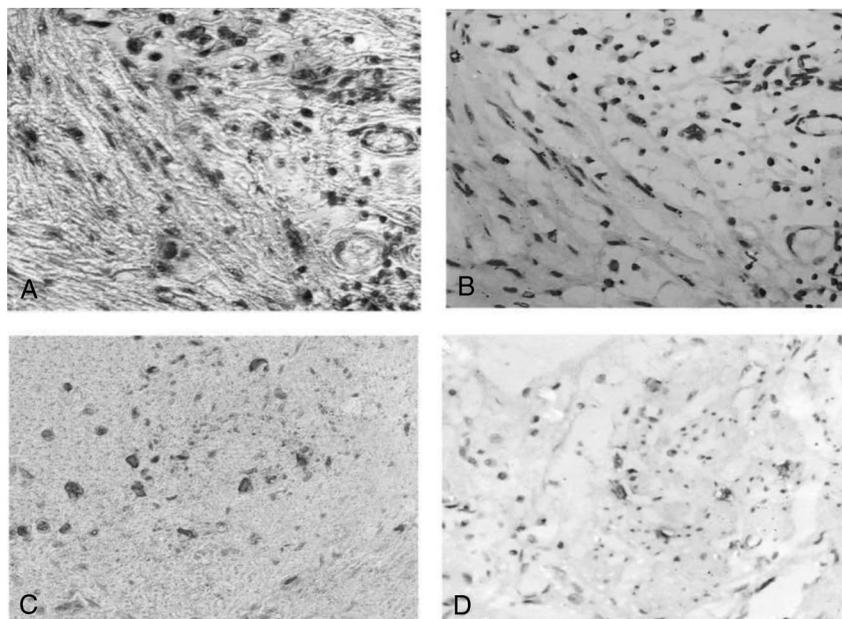


FIGURE 5. A, Mast cells expression (AEC) and (B) IgE expression (DAB) in the same area of consecutive section in allergic throat tissue. C, Mast cells expression (AEC) and (D) IgE expression (DAB) in the same area of consecutive sections in allergic intestine tissue.

In the allergic tissues, the number of mast cells and the amount of IgE expression varied in the different tissue types ($P < 0.05$), whereas the mast cell degranulation rate showed no differences ($P > 0.05$; Table 4). These 3 factors did not differ by sex or age ($P > 0.05$; Table 5).

Correlation Between Marker Expression (Mast Cells, Mast Cell Degranulation, and IgE) and PMI in the Allergic Group

In lung tissues, the mast cell number ($R = -0.446$, $P < 0.05$) and its degranulation rate ($R = 0.566$, $P < 0.01$), but not IgE-positive cells, were significantly and positively correlated with PMI. In the intestine tissues, IgE positivity was inversely related to the time of death ($R = -0.742$, $P < 0.01$), whereas no relationship was found in the 2 other indicators. In comparison, in allergic laryngeal tissues, no correlation with the time of death was found in the number of mast cells, rate of mast cell degranulation, or IgE-positive cells. All the correlation data are shown in Table 6.

DISCUSSION

Anaphylaxis is difficult to diagnose in autopsy, and scholars have sought practical and specific biomarkers for postmortem diagnosis of anaphylactic death. Mast cell degranulation and the release of mediators play essential roles in anaphylactic reactions. Tryptase, a protein stored in the secretory granules in mast cells, is an indicator of anaphylactic reactions. Research proves that

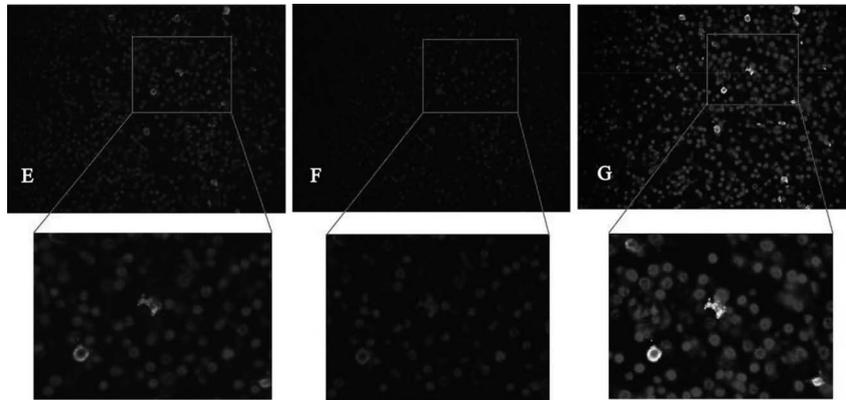


FIGURE 6. E, Representative micrograph of mast cells (tryptase: green) in allergic throat tissue. F, Immunoglobulin E–positive cells in allergic throat tissue. G, Double-label immunofluorescence shows that co-localization of mast cells (tryptase) with IgE is expressed in allergic throat tissue.

tryptase measurements are valuable in indication of anaphylactic sudden unexpected or unwitnessed deaths.^{4,9,10} Sun et al¹⁴ performed a meta-analysis of systematic reviews and noted that high serum tryptase concentrations ($>30.4 \mu\text{g/L}$) would be a good indicator for the postmortem diagnosis of anaphylaxis. A high level of tryptase is considered as an indicator of mast cell activation in anaphylaxis.¹² Nevertheless, the tryptase levels detected in victims experiencing anaphylaxis may occasionally be normal, but an elevated tryptase level may be misleading because it can also be seen in patients with trauma, asphyxia, atherosclerosis, cardiovascular disease, or heroin overdose.^{15–18} Moreover, a study showed a positive correlation between tryptase concentration and increasing PMI.¹¹ In contrast, another study reported that varying PMI had little influence on tryptase levels.¹⁹ In 2015, Sravan et al²⁰ reported an anaphylactic death with serum tryptase level significantly declining with increasing PMI and assumed that the reduction may have resulted from the degradation of degranulated

mast cell tryptase after anaphylactic death.²¹ Immunohistochemical methods used to examine tissues after anaphylactic deaths may aid in postmortem diagnosis.²² An accumulation of mast cells was recently found in the spleen of anaphylactic deaths,¹⁷ and the level of tryptase was greatly enhanced in anaphylactic pulmonary tissues.^{23,24} Moreover, Luongo et al²⁵ showed an increased number of mast cells in allergic laryngeal tissue, and Unkrig et al¹⁶ reported a case of suspected food anaphylaxis and proposed that the high expression and degranulation of mast cells in the intestine, lung tissues, and throat mucosa can offer instructions for postmortem diagnosis of anaphylactic shock. In our research, the allergic samples showed significantly higher numbers of mast cells than did the controls in the throat, lung, and intestine. This result is partly distinct from the research by Edston¹⁸ in 2013, who suggested that increased numbers of mast cells and eosinophil granulocytes in the spleen, but not in the lung, can aid in diagnosis of anaphylactic death. The differences may be explained

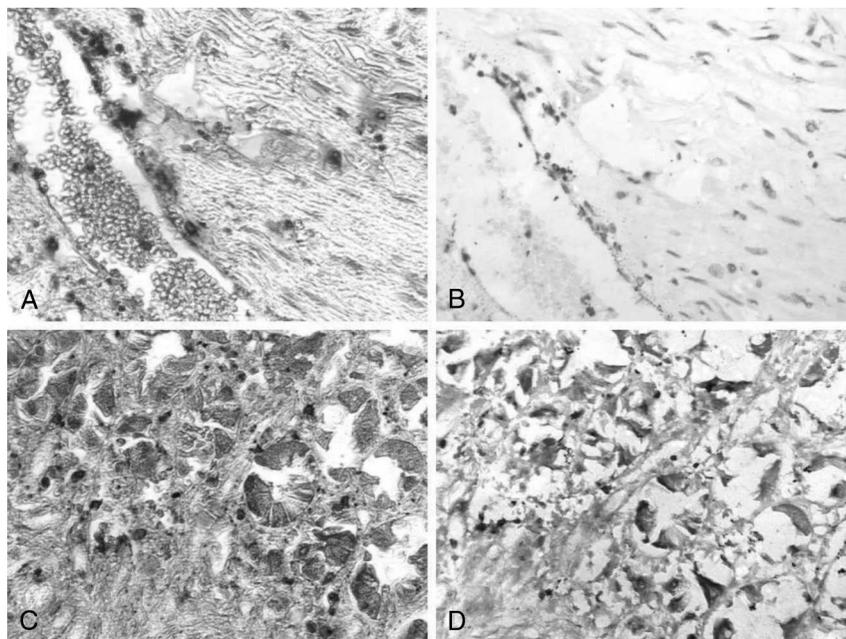


FIGURE 7. A, MC expression and degranulation (AEC) in larynx tissue of whom dead from electric shock and (B) the negative expression of IgE (DAB) in the same area. C, MC expression and nondegranulation (AEC) in larynx tissue of whom dead from heart attack and (D) negative expression of IgE (DAB) in the same area.

TABLE 4. The Relational Detection Results of MC, Degranulation Ratio of MC, and IgE in 3 Different Tissue Types

| Tissue | N | MC | P | Degranulation Ratio | P | IgE | P |
|-----------|----|-----------|------|---------------------|------|-----------|------|
| throat | 20 | 5.66±0.40 | 0.02 | 0.65±0.037 | 0.52 | 4.42±0.57 | 0.01 |
| Lung | 20 | 5.38±0.33 | | 0.70±0.042 | | 2.13±0.42 | |
| intestine | 20 | 7.54±0.85 | | 0.68±0.035 | | 3.80±0.72 | |

firstly by the difficulty of precisely counting the mast cells in lung tissues because of the irregular architecture and secondly by the different PMIs among the cases, because a portion of mast cells may have degranulated and become invisible with increasing PMI.¹⁸

In a tissue where mast cells already have antigen-specific IgE bound to FcεRI, re-exposure to the original antigen can lead to the crosslinking of FcεRI-bound IgE and the aggregation of FcεRI in the cell surface. Consequently, mast cells are triggered to start complex signaling events that ultimately cause the secretion and release of a diverse group of mediators. These bioactive products contain some stored preformed mediators in the intracellular cytoplasmic granules (eg, tryptase, chymase, histamine, 5-ht), newly synthesized mediators (eg, PGD₂, LTC₄, LTB₄, and LTD₄), and certain cytokines. Eventually, the catastrophic immune responses that can rapidly cause death are prompted. Unfortunately, as the most direct objective indicator for mast cell activation, increases in mast cell degranulation in human autopsy tissues after allergic sudden death have rarely been statistically analyzed. With IHC staining, Unkrig et al¹⁶ marked the mast cells with antitryptase antibodies in one anaphylactic death and noted that tryptase-positive particles scattered as “starry sky-like or yard-like distributed” material in the activated mast cells.¹⁶ Our study showed that both the number of mast cells and the degranulation rate were significantly higher in the anaphylactic death group than in the control group.

These results do not exclude the possibility of tryptase passive diffusion after mast cell autolysis in allergic and nonallergic tissues after death, but the allergic samples showed significantly higher mast cell degranulation rates than did the controls. This result implies that the high expression and degranulation of mast cells play a major part in the changes of anaphylactic shock and may be helpful in the diagnosis.

Immunoglobulin E, also a key factor in anaphylactic reaction, has attracted the attention of researchers.^{26–28} However, some studies have found that serum IgE measurement has limitations because of its short half-life in the blood and the lack of value for the differential diagnosis of anaphylaxis from other allergic diseases (eg, asthma). In this study, we examined and quantified the IgE expression in human tissues and involved some of the autopsies with a PMI for 1 month. Our results show that the number of IgE-positive cells is significantly higher than that in the nonallergic group. This finding suggests that IgE content is more stable in tissue than in the serum and indicates that the detection of IgE content in tissues is important for the forensic diagnosis of sudden death from allergy. Unexpectedly, no IgE-positive expression was found in 5 allergic lung tissues and 1 allergic throat tissue of the 20 total cases. The cause is unclear, but we consider that the short half-life makes IgE degrade in the loosened structures of lung and throat tissues during the PMI. Another possibility may be that

TABLE 5. Relationship Among Clinical Pathology Parameters and Number of Mast Cells, the Mast Cell Degranulation Ratio, and IgE-Positive Cells in Allergic Tissue

| Tissues | Variables | Cases | Mast Cell, n/hp* | P | Mast Cell Degranulation Rate | P | IgE, n/hp* | P |
|-----------|-----------|-------------|------------------|-------------|------------------------------|-------------|-------------|-------|
| Throat | Sex | | | 0.09 | | 0.36 | | 0.547 |
| | Male | 9 | 6.40 ± 0.66 | | 0.69 ± 0.07 | | 4.03 ± 0.99 | |
| | Female | 11 | 5.05 ± 0.42 | | 0.63 ± 0.03 | | 4.75 ± 0.66 | |
| | Age, y | | | 0.064 | | 0.994 | | 0.588 |
| | <20 | 2 | 3.40 ± 1.10 | | 0.65 ± 0.08 | | 2.60 ± 0.60 | |
| | 20–50 | 14 | 5.61 ± 0.47 | | 0.66 ± 0.04 | | 4.64 ± 0.59 | |
| Lung | >50 | 4 | 6.93 ± 0.40 | | 0.65 ± 0.11 | | 4.58 ± 2.05 | |
| | Sex | | | 0.971 | | 0.911 | | 0.151 |
| | Male | 9 | 5.39 ± 0.44 | | 0.70 ± 0.08 | | 1.06 ± 0.41 | |
| | Female | 11 | 5.36 ± 0.50 | | 0.71 ± 0.04 | | 2.27 ± 0.65 | |
| | Age, y | | | 0.114 | | 0.183 | | 0.330 |
| | <20 | 2 | 6.80 ± 0.10 | | 0.66 ± 0.08 | | 3.60 ± 0.70 | |
| Intestine | 20–50 | 14 | 4.94 ± 0.40 | | 0.75 ± 0.04 | | 1.45 ± 0.47 | |
| | >50 | 4 | 6.18 ± 0.42 | | 0.55 ± 0.14 | | 1.75 ± 1.16 | |
| | Sex | | | 0.635 | | 0.835 | | 0.278 |
| | Male | 9 | 7.08 ± 0.95 | | 0.67 ± 0.03 | | 2.98 ± 0.82 | |
| | Female | 11 | 7.92 ± 1.36 | | 0.68 ± 0.06 | | 4.52 ± 1.11 | |
| | Age, y | | | 0.108 | | 0.082 | | 0.093 |
| <20 | 2 | 4.50 ± 2.40 | | 0.46 ± 0.18 | | 6.90 ± 1.90 | | |
| 20–50 | 14 | 7.06 ± 0.90 | | 0.73 ± 0.04 | | 2.80 ± 0.47 | | |
| >50 | 4 | 10.8 ± 2.11 | | 0.62 ± 0.38 | | 5.73 ± 2.91 | | |

TABLE 6. Correlation Between the Mast Cells, Mast Cell Degranulation, IgE Expression, and the PMI in the Allergic Group

| PMI | Tissues | Mast Cell | | Mast Cell Degranulation | | IgE | |
|-----|-----------|-----------|-------|-------------------------|-------|--------|--------|
| | | R | P | R | P | R | P |
| | Throat | 0.129 | 0.589 | 0.342 | 0.139 | 0.081 | 0.735 |
| | Lung | -0.446 | 0.049 | 0.566 | 0.009 | -0.131 | 0.582 |
| | Intestine | 0.024 | 0.920 | 0.316 | 0.175 | 0.000 | -0.742 |

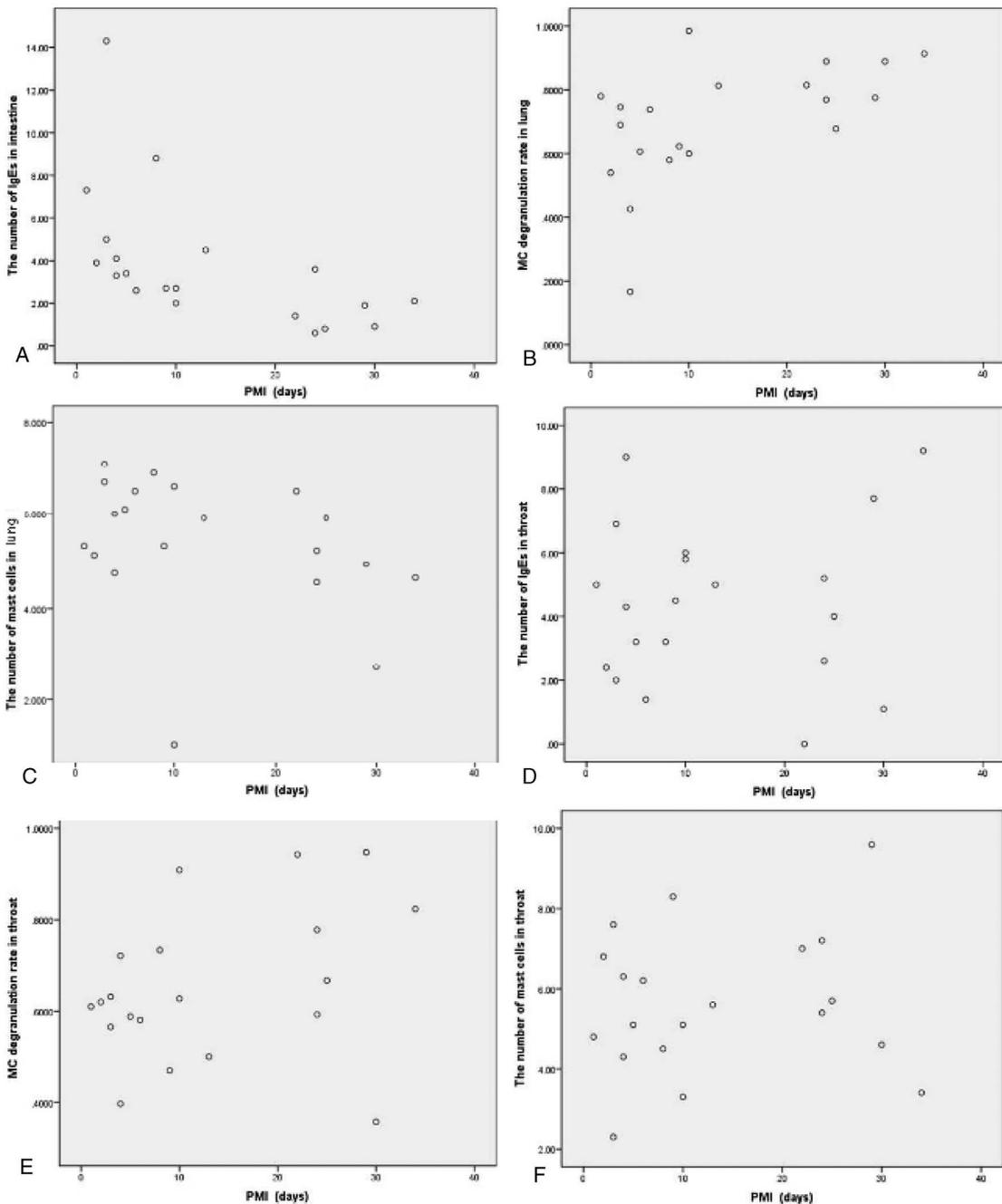


FIGURE 8. A, Correlation between the PMI and the counting result of IgE-positive cells in allergic intestinal tissue ($R = -0.742, P < 0.01$). B and C, Correlation between PMI and mast cells number ($R = -0.446, P < 0.05$) and its degranulation ratio ($R = 0.566, P < 0.01$) in allergic lung tissue. D–F, No correlation between PMI and the number of mast cells, the number of IgE-positive cell, or mast cell degranulation rate in allergic laryngeal tissue.

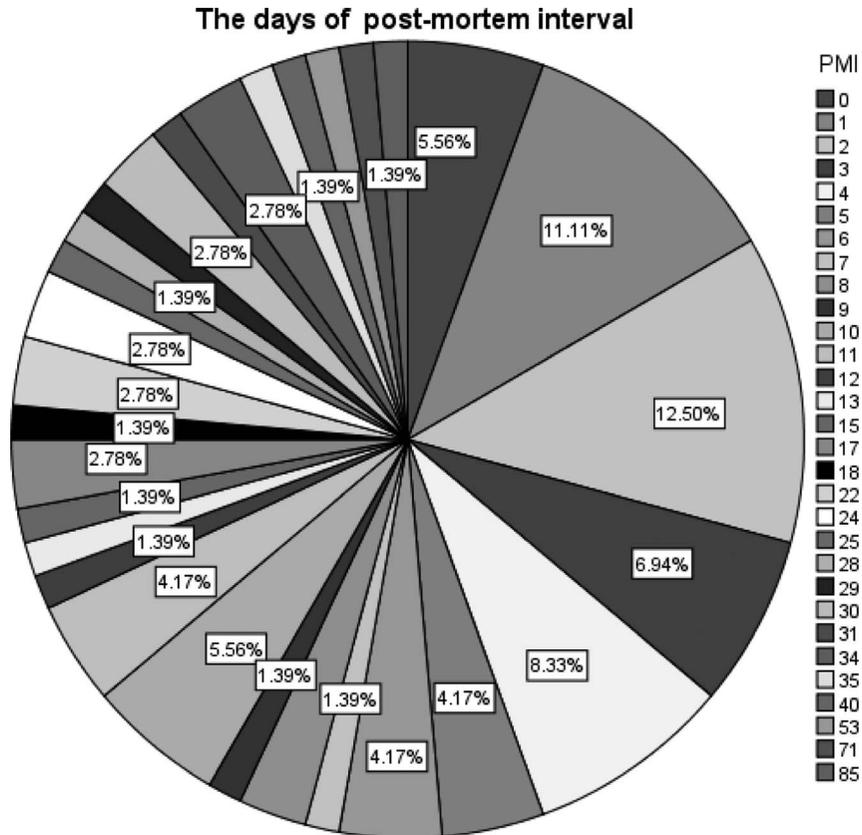


FIGURE 9. The PMI of the cases ranged from days to several weeks.

mast cells are activated by a non-IgE pathway in the negative cases. As reported, IgG may mediate the release of activated mediators from mast cells in tissues.

We performed dual immunity group dyeing to observe the co-localization of mast cells and IgE. Most of the IgE was found in the cytoplasm and membrane of mast cells, and most of the mastocytes with IgE were in a state of degranulation. Conversely, in the controls, IgE was almost negatively expressed whether the MC was activated. This may be direct evidence that the sequence of events that lead to mast cell activation is IgE-mediated reactions in cases of anaphylaxis. To further corroborate these findings, we performed double IF staining and found consistent results with previous observations. Here, we considered double IHC as a simple cost-effective method to observe the co-location of important mediators and to investigate their relationship. In addition, post-mortem examinations may be performed from hours to weeks after death, so forensic diagnoses are occasionally disturbed by autolysis, tissue putrefaction, or effective ingredient degradation. We preliminarily attempted to explore the correlation between the expression of these important mediators and the PMI. Results showed that the IgE levels decreased in intestinal tissues as the PMI increased and that the mastocyte degranulation rate increased in the lung. The mechanism is not completely understood, but it likely occurs because the intestine produces many different digestive enzymes to help IgE degradation, which is characteristic of instability, and because the loose lung tissue is rich in blood vessels, with the active matter spreading to contribute to mast cell degranulation. Interestingly, no significant association was found between the expression of these mediators and increasing PMI in the larynx. The contents of these mediators in the larynx were

more stable than in other tissues. The exact reason is unclear, but it may be that laryngeal tissue has fewer blood vessels and less digestive enzymes than do other tissues of the body. Our results may be inaccurate to some extent because of a lack of more autopsy specimens and because of the differences in the ambient temperature and microbe distribution of the individuals. Hence, further study is required to explore the relationship between tissue antigen stability and PMI in forensic science. Figures 8 & 9

CONCLUSIONS

We demonstrate a significant increase in mast cell degranulation accompanied by an increase in IgE levels in throat tissue after allergic sudden death. Elevated mast cell degranulation combined with increased IgE levels is a reliable biomarker for forensic diagnosis of the bodies because of IgE-mediated allergic sudden death.

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