Original Research Report

EXPRESSIONS OF β-TRYPTASE AND CHYMASE IN LUNG MAST CELLS DUE TO ANAPHYLACTIC SHOCK THROUGH HISTOPATHOLOGICAL APPEARANCE AT DIFFERENT POST-MORTEM INTERVALS

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ABSTRACT

Anaphylactic shock is a hypersensitivity response, a commonly type I hypersensitivity involving immunoglobulin E (IgE). It is caused by an antigen-antibody reaction that occurs immediately after a sensitive antigen enters the circulation. Anaphylactic shock is a clinical manifestation of anaphylaxis that is distributive shock, characterized by hypotension due to sudden blood vessel vasodilation and accompanied by a collapse in blood circulation that can result in death. β-tryptase and mast cell chymase expressions in the lungs of histopathological specimens that had experienced anaphylactic shock were examined at different post-mortem intervals in this study. A completely randomized design (CRD) method was employed by collecting lung samples every three hours within 24 hours of death, and then preparing histopathological and immunohistochemical preparations. The mast cell tryptase and chymase expressions were counted and summed up in each field of view, and the average was calculated to represent each field of view. The univariate analysis yielded p-values of 0.008 at the 15-hour post-mortem interval, and 0.002 at the 12-hour post-mortem interval. It was concluded that tryptase and chymase can be utilized as markers of anaphylactic (non-anaphylactoid) shock in the lungs.

Keywords: Anaphylactic shock; β-tryptase; chymase; post-mortem interval; mortality

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Highlights:

1. The post-mortem interval is related to tryptase and chymase expressions in anaphylactic shock incidence.
2. Forensic experts can utilize tryptase and chymase as markers of anaphylactic (non-anaphylactoid) shock that occurs in the lungs.

INTRODUCTION

Anaphylaxis is literally derived from the Greek words "*ana*" that means "return" and "*phylaxis*" that means "protection" (McLendon & Sternard 2022). The immune response that should protect against a specific disease, also known as prophylaxis, actually damages the tissue in the case of anaphylaxis. In other words, it does the opposite of what it should, which is why it is referred to as anti-phylaxis or anaphylaxis (Smith 2015). Anaphylaxis is a type of distributive shock that causes hypotension due to abrupt blood vessels vasodilation, followed by a collapse in blood circulation that can lead to death (Kounis et al. 2013). The clinical manifestation of anaphylaxis is anaphylactic shock (Poziomkowska-Gęsicka & Kurek 2020).

Anaphylactic shock is a hypersensitivity response, frequently type I hypersensitivity, involving immunoglobulin E (IgE). It is triggered by an antigen-antibody reaction shortly after a sensitive antigen enters the circulation (Reber et al. 2017). Allergens or hazardous agents can cause type I hypersensitivity, which may be more dangerous in some people (Abbas et al. 2022). When asthmatic

patients are exposed to these substances, it can cause IgE-mediated hyper-sensitivity that can be exacerbated by the presence of airway resistance (Yudhawati & Krisdanti 2019). In children with allergic rhinitis, house dust mite allergies can trigger anaphylactic episodes (Endaryanto & Nugraha 2022). People with medical disorders and medication dependence are required to receive immunotherapy, which may minimize the risk of anaphylactic shock (Shinee et al. 2019).

Anaphylactic shock has become more common every year. According to epidemiological data, there are 50-2,000 episodes per 100,000 patients (0.5-2%). The prevalence of anaphylactic shock in animals is still unknown (Shmuel & Cortes 2013). However, several cases of anaphylactic shock had been recorded in dogs (Bosmans et al. 2014) and calves (Choi et al. 2019) as a result of antibiotic medication. Non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics are the most common causes of anaphylaxis (Rengganis 2016). Post-mortem diagnosis of anaphylactic shock is a challenging task for pathologists today. The method for post-mortem examination of anaphylactic shock can use numerous mediators that play a role in its occurrence. However, it still needs development for its use in different situations.

MATERIALS AND METHODS

The study used a completely randomized design (CRD) approach to collect lung samples and determine the minimal sample size, as suggested by (Stefanski et al. 2018). The formula employed was $(n-1)(t-1)\geq 15$, which would require at least two rabbits if the result was $n\geq 2$. Histopathological and immunohistochemical preparations were conducted every three hours within the first 24 hours after death. The samples should be assessed quantitatively according to the modified Remmele method (Bayo 2019).

The 24-hour observation time and the observation organ (i.e., the lungs) were the independent variables in this study. Univariate analysis was used to determine the results in the form of statistical measures, tables, and graphs for each variable. Univariate analysis is used to examine each variable in the research findings (Canova et al. 2017). The purpose of univariate analysis is to summarize the measurement data set in such a way that the data set becomes usable information. The summary can be composed of statistics, tables, or graphs and is generated for each variable.

The dependent variables were the amount of immunoreactive mast cells and the immunohisto-chemical appearance of the lungs every three hours after death to assess detectable β-tryptase and chymase protein expressions in different post-mortem intervals (PMIs). These were the variables that resulted from the treatment and would be examined in this study. The control variables in this study were the species, sex, age, and body weight of the experimental animals, as well as the dose of the anaphylactic reaction agents and environmental conditions. As suggested by Hanlon & Vanderah (2010), the anaphylactic reaction agents were 1 mg of ovoalbumin and 0.5 ml of Freund's adjuvant.

RESULTS

A statistical analysis employing a multivariate repeated measure test revealed that there was an effect of the treatment on the expression of mast cell tryptase in the lungs, which was observed at different post-mortem intervals. Table 1 shows the expression of mast cell tryptase in the lungs of the experimental animals.

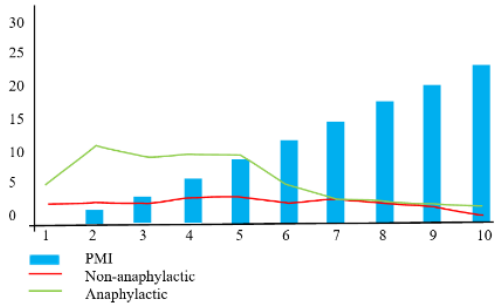

Figure 1. Lung mast cell tryptase of the anaphylactic and non-anaphylactic groups according to the PMIs.

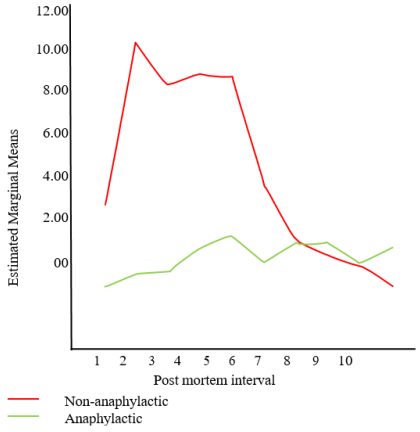

Figure 2. Interaction curve plots of the treatment groups with different PMIs.

Table 1. Data on mast cell tryptase in the lungs of the anaphylactic and non-anaphylactic groups.

PMI	Group	Average	SD
0 h	Non-anaphylactic	1.7500	1.06066
	Anaphylactic	4.8000	0.42426
	Total	3.2750	1.88038
1 h	Non-anaphylactic	2.2000	0.42426
	Anaphylactic	10.9000	0.28284
	Total	6.5500	5.03157
3 h	Non-anaphylactic	2.2500	1.060666
	Anaphylactic	9.2800	1.86676
	Total	5.7650	4.24385
6 h	Non-anaphylactic	3.1000	0.28284
	Anaphylactic	9.7000	1.69706
	Total	6.4000	3.93785
9 h	Non-anaphylactic	3.4500	0.07071
	Anaphylactic	9.6000	1.27279
	Total	6.5250	3.62618
12 h	Non-anaphylactic	2.4500	0.49497
	Anaphylactic	5.4700	0.38184
	Total	3.9600	1.78056
15 h	Non-anaphylactic	3.3000	0.14142
	Anaphylactic	3.3000	0.14142
	Total	3.3000	0.11547
18 h	Non-anaphylactic	2.7000	0.14142
	Anaphylactic	3.2000	0.00000
	Total	2.9500	0.30000
21 h	Non-anaphylactic	2.3000	0.28284
	Anaphylactic	2.3000	0.28284
	Total	2.3000	0.23094
24 h	Non-anaphylactic	1.6000	0.14142
	Anaphylactic	2.9000	0.00000
	Total	2.2500	0.75498

Observation at the 3-hour post-mortem interval showed that the quality of positive outcomes differed between the non-anaphylactic and anaphylactic groups. Mast cells, which were found in the submucosal connective tissue and interstitial tissue of the lungs, appeared to be proliferating in some fields of view. Tryptase expression was detected in the bronchioles and veins at the 3-hour post-mortem interval.

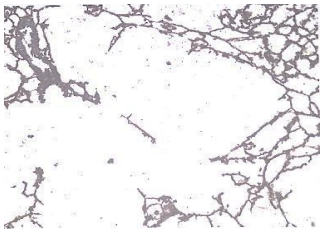

Figure 3. Immunohistochemical staining showed alveoli tissue in anaphylactic rabbits with emphysema (100x magnification of the lung mast cell tryptase).

At the 9-hour post-mortem interval, the non-anaphylactic and anaphylactic groups showed a difference in the quality of the positive results. There was a moderate accumulation of transudate fluid in the lumen of the alveoli and bronchioles in both the non-anaphylactic and anaphylactic groups. Bronchiolar integrity appears incomplete with negative immunoreactivity. The mast cell granules in the non-anaphylactic group were clearly visible intracytoplasmically.

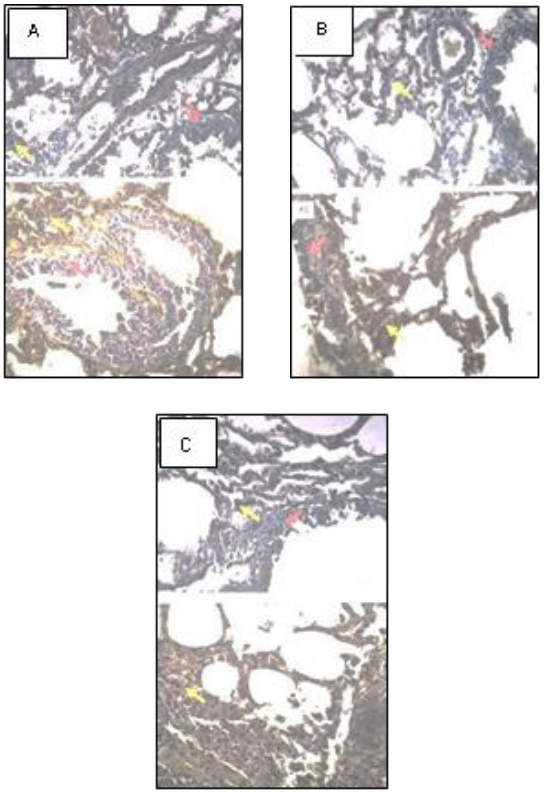

Figure 4. Immunohistochemical staining of mast cell tryptase at 0 h PMI (A), 1 h PMI (B), and 3 h PMI (C).

Red arrows in Figure 4 indicate negative immunoreactivity in the bronchioles. Yellow arrows indicate the mast cells. The anaphylactic group showed positive and proliferative immunoreactivity (400x magnification of the lung mast cell tryptase).

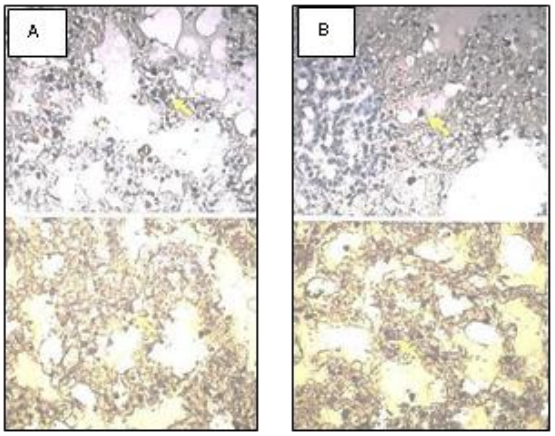

Figure 5. Immunohistochemical staining of mast cell tryptaseat 6 h PMI (A) and 9 h PMI (B).

Yellow arrows indicate the mast cells. The non-anaphylactic group showed positive immuno-reactivity in the mast cell granules (400x magnification of the lung mast cell tryptase.

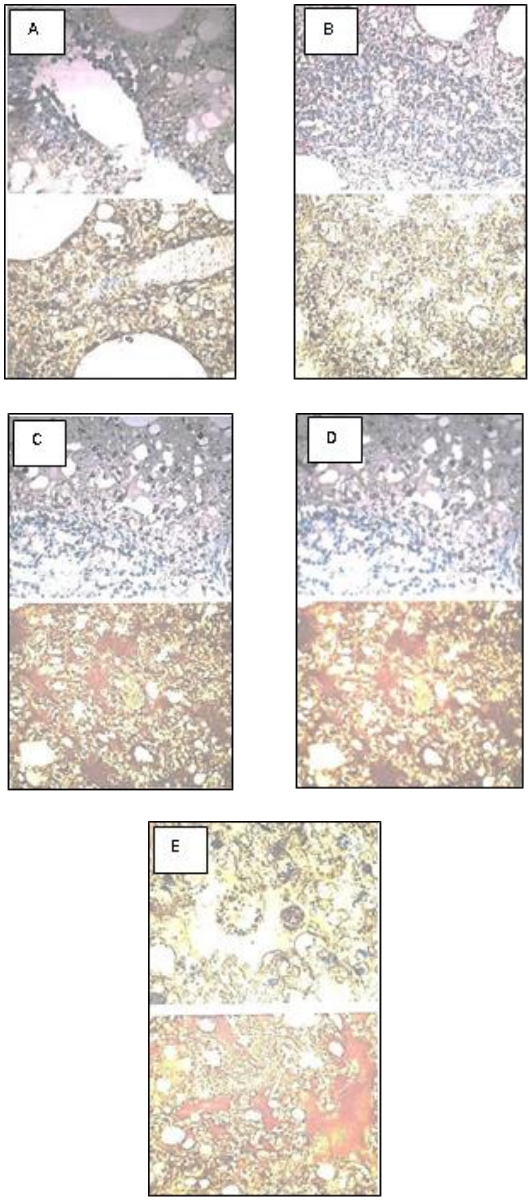

Figure 6. Immunohistochemical staining of mast cell tryptase at 12 h PMI (A), 15 h PMI (B), 18 h PMI (C), 21 h PMI (D), and 24 h PMI (E).

The bronchioles appeared damaged, and plasma had accumulated in the alveoli. The non-anaphylactic group showed weak positive immunoreactivity (400x magnification of the lung mast cell tryptase).

Table 2. Data on mast cell chymase in the lungs of the anaphylactic and non-anaphylactic groups.

PMI	Group	Average	SD
0 h	Non-anaphylactic	2.1500	0.77782
	Anaphylactic	5.0000	0.28284
	Total	3.5750	1.71343
1 h	Non-anaphylactic	2.6000	0.00000
	Anaphylactic	7.0500	1.34350
	Total	4.8250	2.68375
3 h	Non-anaphylactic	1.3500	0.07071
	Anaphylactic	7.2500	1.34350
	Total	4.3000	3.49380
6 h	Non-anaphylactic	2.4500	0.07071
	Anaphylactic	6.4300	0.52326
	Total	4.4400	2.31799
9 h	Non-anaphylactic	4.5600	1.04652
	Anaphylactic	6.4900	1.08894
	Total	5.5250	1.41491
12 h	Non-anaphylactic	2.6100	0.43841
	Anaphylactic	5.0000	0.14142
	Total	3.8050	1.40526
15 h	Non-anaphylactic	1.8300	0.01414
	Anaphylactic	3.2800	0.05657
	Total	2.5550	0.83728
18 h	Non-anaphylactic	1.3100	0.21213
	Anaphylactic	2.6400	0.00000
	Total	1.9750	0.77758
21 h	Non-anaphylactic	1.0800	0.16971
	Anaphylactic	1.9600	0.50912
	Total	1.5200	0.59509
24 h	Non-anaphylactic	0.8000	0.00000
	Anaphylactic	0.8700	0.35355
	Total	0.8350	0.20809

The quality of positive results in the non-anaphylactic and anaphylactic groups differed during the 15-hour post-mortem interval. In the non-anaphylactic and anaphylactic groups, there was a moderate accumulation of transudate fluid in the lumen of the alveoli and bronchioles. The bronchiolar integrity appears incomplete, with just a faint positive immunoreactivity. The non-anaphylactic group's mast cell granules were clearly visible intracytoplasmically.

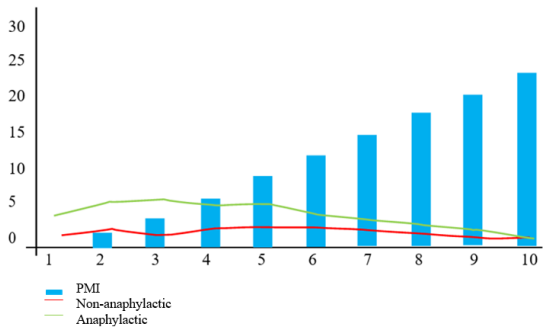

Figure 7. Descriptive curves of the lung mast cell chymase at different post-mortem intervals.

Table 3. Results of the univariate analysis of the lung mast cell chymase.

	Contrast	Error
Sum of squares	6.366	0.029
df	1	2
Mean square	6.366	0.051
F	433.590	
Sig.	0.0020	
Partial eta squared	0.995	

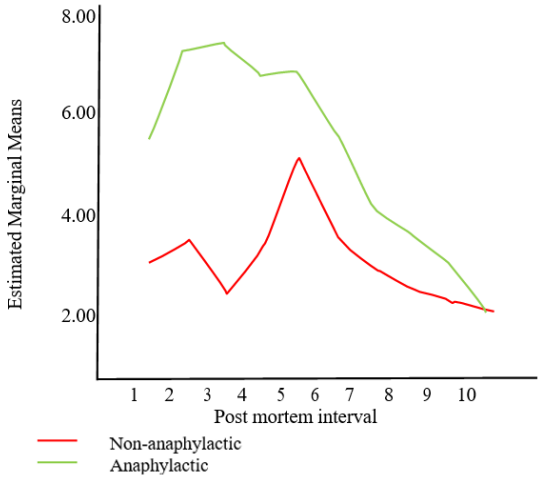

Figure 8. Estimated marginal means of mast cell tryptase.

A statistical analysis utilizing a multivariate repeated measure test revealed that there was an effect of the treatment on the mast cell chymase expression in the laryngeal organs, which was observed at different post-mortem intervals. Table 2 exhibits the mast cell chymase expression in the lungs of the experimental animals.

DISCUSSION

This study analyzed the expressions of lung mast cell β-tryptase and chymase due to anaphylactic shock, which were observed at different post-mortem intervals. This research employed rabbits (*Oryctolagus cuniculus*) that provided three types of specimens, i.e., the lungs, larynx, and heart.

A previous experimental laboratory research project employed a randomized block design (RBD) with equal subjects and a time series. The purpose of the research was to prove the relationship between the post-mortem interval and changes in immuno-histochemical features by analyzing theexpression of mast cell β-tryptase and chymase proteins in rabbit lung organs that experienced anaphylactic shock. Blood vessels of the rabbits (*Oryctolagus cuniculus*) were used as a scientific input to establish a precedent for mortality cases due to anaphylactic shock (Turner et al. 2017). The experimental animals had a one-week acclimatization period. Pellets were used for feeding, and drinking water was available ad libitum or without restriction. Clinical pathological examination of the complete blood picture of the rabbits was carried out to assess the presence of infection or other clinical indications that could interfere with the results of the study.

A study found a 25% prevalence of type I hypersensitivity among patients with drug-related hypersensitivity (Isyroqiyyah et al. 2021). Although the prevalence might appear to be low, this could lead to life-threatening consequences. Another study found that medications, such as antibiotics, were the most common cause of anaphylactic reactions (Hasanah et al. 2020).

A forensic expert is required in a variety of situations concerning the body, health, and life. The expert can identify corpses, human body parts, body conditions, time of death, cause of death, and provide a variety of written statements.

Strength and limitations

This study revealed that the expressions of tryptase and chymase at different post-mortem intervals could be utilized by forensic science technicians and experts as markers of anaphylactic shock. However, because this study was conducted in a controlled environment, the results may alter if other conditions are applied.

CONCLUSION

Forensic science technicians and experts can use tryptase and chymase expressions at different post-mortem intervals as anaphylactic shock (non-anaphylactoid) markers in the lungs, with varying mast cell chymase and tryptase production amounts and qualities.

Acknowledgment

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Conflict of interest

None.

Ethical consideration

This study was approved by the Research Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, with the reference

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None.

Author contribution

BAP, IS, and AY contributed in gathering and analyzing the data. All authors contributed to the preparation and approval of the manuscript for publication.

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