

## Comparative histamine levels in antemortem and postmortem wounds in the human skin by fluorescence spectrophotometry

Faridah MN, MD (UKM), MPath(Forensic) and Shahrom AW, DCP (Lond), MRCPath(Forensic)

Forensic Unit, Department of Pathology, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

### Abstract

This paper describes a modified method of quantitative determination of histamine in human skin wounds using fluorescence spectrophotometer. In this study, histamine was used as an indicator to differentiate antemortem from postmortem wounds. Skin samples were obtained from 20 corpses which were brought to Hospital Kuala Lumpur and Universiti Kebangsaan Malaysia for medicolegal autopsy. Sections of human skin were processed biochemically for histamine determination using fluorescence spectrophotometer. Results revealed no significant difference in the histamine content of the antemortem wounds in comparison to postmortem wounds. Based on these results, detection of histamine is not suitable to differentiate antemortem from postmortem wounds.

**Key words:** Histamine; Fluorescence spectrophotometer; Skin; Antemortem wound; Postmortem wound.

### INTRODUCTION

In forensic medical practice, numerous methods for the examination of the vital reaction in wounds have been published. Histamine determination in wounds is a well-known indicator used to determine the vitality of short-term wounds.<sup>1-6</sup> Other natural substances of interest are enzymes such as cathepsin D and trace metals such as calcium, manganese and etc. which are widely distributed in human tissues.<sup>7-11</sup> As human skin is the most susceptible organ to environmental trauma, therefore, it is the most suitable specimen to study. Much of the experimental work has been performed on animals and the results are not directly transferable to man,<sup>7,12-15</sup> therefore human skin is chosen in this study for comparability to previous studies.

### MATERIALS AND METHODS

Extraction and isolation of histamine from tissue samples were carried out according to the method described by Sekardi and Friedberg.<sup>16</sup>

#### Chemicals

Histamine salts was obtained from Sigma Chemical Company (St. Louis, USA). All other chemicals such as ethanol, sodium phosphate powder, hydrochloric acid, sodium hydroxide

salts, O-phthaldialdehyde salts, methanol and citric acid powder were of analytical grade.

#### Treatment of samples

Skin samples 2x2 cm in size, elliptical in shape were taken from wounded areas and from contralateral healthy nonwounded area (postmortem wounds) to serve as controls. The wounds were determined as antemortem by evidence of vitality such as redness and swelling whilst postmortem wounds were wounds inflicted after death. All cases selected in this study consist of victims of motor vehicle accidents. All the subcutaneous fat of samples were removed and the skin samples were labelled and kept in propylene test tubes. They were then weighed and stored at 10°C until used.

#### Preparation of tissue extracts

All the excised skin tissue were dried on a metal tray over a waterbath at a temperature of 37°C for about two weeks. The dried tissues were then cut into small pieces before being pulverized with a tissuemizer. Mortar and pestle were used to quicken the process.

The pulverized skin, 100-300 mg, was subsequently mixed with 50 mg of sodium chloride and 8 ml of 80% ethanol to split the histamine-heparin complex and to extract the

histamine respectively. After 30 minutes, the solution was centrifuged at 1500 rpm for 10 minutes. The supernatant was mixed with 6 ml of 80% ethanol and boiled in waterbath for 3 minutes and then centrifuged at 1500 rpm for 10 minutes. The supernatant was filtered through a filter paper. The alcohol was evaporated from the histamine extract by using a waterbath until 0.5 ml aqueous phase remained. The sample was then mixed with 5 ml of 0.1 mol/L sodium phosphate buffer and histamine was isolated on a cation exchange resin, Dowex 50 WX8, mesh 200-400. Histamine was bound to the matrix of the exchange resin. The accompanying substances were washed off in steps, first, with 5 ml of sodium phosphate buffer, then, with 1 ml bidistilled water and finally, with 5 ml 1N hydrochloric acid.

The histamine bound to the exchange resin was eluted with 3 ml of 4N of hydrochloric acid. The eluate was then diluted with 3 ml bidistilled water. 1.7 ml of this diluted eluate was mixed with 0.8 ml of 5N sodium hydroxide and 0.1 ml

of O-phthaldialdehyde (1% OPT solution in metanol uvasol). After 2 minutes, the fluorescent condensation product of histamine and OPT was stabilised with 0.6 ml of 2 mol/L citric acid. For reagent blank, 1.7 ml of the sample was replaced with 1.7 ml of 2N hydrochloric acid.

#### Analytical method

Analytical measurement of histamine was detected by fluorescence spectrophotometer Hitachi F-2000 (an excitation wavelength 350 nm and a fluorescent of 440 nm).

## RESULTS

### Histamine

For histamine determination, 20 paired skin samples were analysed. The histamine content in antemortem and postmortem wounds are tabulated in Table 1. The null hypothesis for this study is to show that there was no significant difference in histamine content between antemortem and postmortem wounds in the

**TABLE 1: Results of histamine content in human skin from antemortem and postmortem wounds**

Postmortem number	Histamine content in human skin from antemotem samples of less than 6 hours injury. ( $\mu\text{g/g}$ )	Histamine content in human skin from postmortem samples. ( $\mu\text{g/g}$ )
P 1183/96	0.64	0.52
P 1457/96	0.83	1.17
P 1029/96	0.31	0.61
P 108/97	1.32	1.60
P 210/97	1.01	0.85
P 1261/96	0.44	0.18
P 209/97	0.44	0.89
P 1248/96	0.49	0.61
P 1245/96	0.75	0.46
P 205/97	1.85	1.44
P 63/97	0.26	0.00
P 1458/96	0.71	1.03
P 211/97	1.69	0.43
P 1246/96	0.47	0.21
P 1310/96	0.48	0.85
P 107/97	0.30	0.68
P 1182/96	0.29	1.35
P 1432/96	0.35	0.82
P 1441/96	0.11	0.46
P 103/97	1.25	0.53
P 1247/96	0.47	1.10
P 255/98	1.44	2.80
Mean $\pm$ 2SD	0.6110 $\pm$ 0.3739	0.7990 $\pm$ 0.6103

human skin. Paired T-test was performed on this data and the result showed that for the 20 paired samples (n=20) with a degree of freedom 19 (df=19), the 'p-value' was 0.124.

## DISCUSSION

Histamine is one of a group of diverse substances that include the biogenic amines, histamine and serotonin, small peptides, such as the kinins and lipids such as the prostaglandins and the chemically unidentified, slow reacting substance of anaphylaxis (SRS-A). All these substances are released from tissues during injury or inflammatory reactions. Dail and Laidlaw in 1919 had demonstrated that when histamine is applied locally, it produces redness, swelling and oedema. Histologically, there is widening of the capillaries, pooling of blood in these vessels and the exudation of plasma through capillary walls. It also serves as a form of defense against microscopic invaders with the accumulation of blood cells and exudates.<sup>10</sup>

In this particular study, histamine is used as an indicator to determine the age of wounds and this determination has been proved accurate by previous study.<sup>17</sup> Experiments were carried out on 20 pairs of human skin samples taken from antemortem and postmortem wounds and their histamine level were compared. After analysis, it was shown that there is no statistically significant difference of the histamine level in antemortem and postmortem wounds. Knight in 1991 performed a study on histamine and showed that there must be at least more than 50% of histamine content in the vital wound than the postmortem wound to indicate that it is vital. Numerous other studies had shown similar results.<sup>2,3,14,17</sup>

Our results appeared to differ from previous studies. This is attributed to several factors, one of which is the small sample numbers. The study should be expanded further for it to be more representative. The cases under study should be more varied to include not only cases involving road traffic accidents but also cases of hanging, drowning, etc. and possibly categorised by sex, age and race to see if there is any difference in the histamine response to wounding.

The time factor is also a crucial determinant in the wound age of the sample. The antemortem wounds, mostly acquired at road traffic accident, were inflicted probably less than 5 minutes or almost immediately prior to death. Previous studies have shown that for histamine to be demonstrable in measureable amount, there

should be survival of at least 20 to 30 minutes after wounding.<sup>9</sup> In our study, survival after wounding was probably too short to allow time for histamine to rise to measureable levels. Hence, in situations of instantaneous death, histamine level and its detection is not useful in determining whether a wound is antemortem or postmortem.

The cases described in this study were mostly road-traffic accident victims with severe multiple injuries and major brain injuries with substantial blood loss and shock. Raekallio J. in 1966<sup>14</sup> noted an impairment of the local reactivity of the skin in severe multiple injuries and major brain injuries whilst blood loss and shock would reduce the histamine content in the tissues. These factors may cause the skin to be less reactive to the obvious effects of wounding and histamine may not accumulate in detectable amounts. This will greatly affect the end result.

In about less than half (13 out of 20 specimens) of the postmortem wounds, there were raised histamine levels. This is in contradiction to the findings of previous researchers. To explain this phenomenon, Sivaloganathan in 1982<sup>18</sup> has found that after death, there is some degradation of histamine in the body which gives rise to a false increase in histamine content in postmortem wounds.

In conclusion, histamine detection to differentiate between antemortem and postmortem wounds is not suitable for use in our centre. However, the analytical method of determining histamine by using fluorescence spectrophotometer has been reported to be precise and sensitive<sup>13,16</sup> as the cation exchange resin is able to remove many interfering substances such as spermidine, spermine and histidine which may affect the end results.

## REFERENCES

1. Betz P. Histological and enzyme histochemical parameters for the age estimation of human skin wound. *Int J Leg Med* 1994;107: 60-8.
2. Fatteh A. Distinction between antemortem and postmortem wounds. *J Forensic Sci* 1965; 11:17.
3. Fazekas IG, Viragos-Kis. Der Gehalt Der Erhangongfurchen An Freim Histamin Als vitale Reaktion. *Dtsch. Z. Ges Gerichtl Med.* 1965; 56: 250-60.
4. Fernandez P *et al.* Biochemical diagnosis of the intravital origin of skin wounds. *Forensic Sci Int* 1994; 68: 83-9.
5. Raekallio J. Determination of the age of wounds by histochemical and biochemical methods. *Forensic Sci Int* 1972; 1: 3-16
6. Zhong FC, Zhen ZJ. Localization and quantification

- of histamine in injured skin as a parameter for the timing of wounds. *Forensic Sci Int* 1991; 51(2): 163-71.
7. Girela E *et al.* Postmortem stability of some markers of intra-vital wounds. *Forensic Sci Int* 1989; 40(2): 123-30.
  8. Hernandez-Cueto *et al.* Study of cathepsin A, B and D activities in the skin wound edges. Its application to the differential diagnosis between vital and postmortem wounds. *Forensic Sci Int.* 1987; 35(1): 51-60.
  9. Kahlson G, Rosengren E. New approaches to the physiology of histamine. *Physiol Rev* 1968 (1):155-96.
  10. Knight B. *Forensic Pathology*. 1<sup>st</sup> ed., Butler & Tanner Ltd., Frome and London.. 1991, p. 152-3.
  11. Shahrom AW, *et al.* Kajian perbezaan luka antemortem dan postmortem menggunakan kation dan hasilan peroksidasi lipid. Laporan Akhir Penyelidikan: IRPA. 1992-1995.
  12. Maeno Y *et al.* A study on the vital reaction in wounded skin: Simultaneous determination of histamine and polyamines in injured rat skin by high performance liquid chromatography. *Forensic Sci Int* 1990; 46(3): 255-68.
  13. Shore PA, Burkhalter A, Cohn Jr VH. A method for the fluorometric assay of histamine in tissues. *J Pharmacol Exp Ther* 1959; 127: 182-96.
  14. Raekallio J. Enzyme histochemistry of vital and postmortem skin wounds. *J Forensic Med.* 1966; 13(3): 85-90.
  15. Raekallio J, Makinen PL. Histamine content as a vital reaction-experimental studies. *Zacchia* 1970;45: 403-14.
  16. Sekardi L, Friedberg KD. A simplified method for the extraction and isolation of histamine from whole blood, plasma and tissues for fluorometric histamine analysis. *Pharmacology* 1982; 24(1): 45-56.
  17. Raekallio J. Timing of wounds – an introductory review. *Ann Acad Med Singapore* 1984; 13(1): 77-84.
  18. Sivaloganathan S. Antemortem injury or postmortem? Diagnosis using histamine as a marker. *Med Sci Law* 1982; 22: 119-25.