IS IT ANTEMORTEM OR POSTMORTEM BLOOD?

By

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ABSTRACT

Bloodstains examination is required in many fields in medicolegal practice. This study was carried out to discriminate between antemortem (AM) and postmortem (PM) bloodstains. The method used depends on determination of the level of human myoglobin (by radio-immunoassay) as well as the levels of adenosine triphosphate (ATP), xanthine and uracil (by HPLC) in the bloodstains. The study revealed a highly statistically significant elevation of myoglobin levels in PM than AM bloodstains. ATP was found in high concentrations in all AM bloodstain samples and could not be detected in any of the PM bloodstain samples. On the other hand, xanthine and uracil were present in considerable concentrations in all the PM bloodstain samples and were not detected in any of the AM bloodstain samples. These results were independent on the age of the stain, the postmortem interval and the cause of death. It is suggested that determination of the above mentioned parameters could be of help in discrimination between antemortem and postmortem bloodstains in medicolegal practice.

INTRODUCTION

Blood evidence can be associated with different kinds of crimes, where bloodstains examination is usually required. The investigations in such cases aim mostly at identification, individualization and reconstruction (Polson et al., 1985).

It is sometimes necessary and crucial to identify bloodstains as antemortem or postmortem. Although discrimination of postmortem (PM) from antemortem (AM) blood is an important aspect in bloodstain analysis, yet few studies were reported on this subject (Takatsu et al., 1991).

Myoglobin is a single polypeptide chain of 153 amino acids and molecular mass of approximately 17,500 dalton. It is found only in striated skeletal and cardiac muscles. Its plasma level is kept low because of its rapid excretion and clearance by the kidneys (renal

threshold : 20mg/100ml plasma) (Kagen, 1983).

The nucleotides are important intracellular molecules of low molecular weight that participate in a wide variety of biochemical processes. The best known role of them is to serve as the monomeric precursors of RNA and DNA. There are also the free nucleotides that are not an integral part of nucleic acids, e.g adenosine triphosphate (ATP). It is the source of high energy phosphate for nearly every energy requiring reaction in the cells (Martin et al., 1987).

Breakdown of either RNA or DNA releases nucleotides which are further degraded to nucleosides then to free purine and pyrimidine bases. In the living organisms, the major pyrimidine bases found in the nucleotides are cytosine, thymine and uracil, while the major purine bases are adenine and guanine. There are also other purine bases that occur as intermediate in the metabolism of adenine and guanine, which are hypoxanthine and xanthine (Martin et al., 1987).

The aim of this work was to investigate a method for discrimination between antemortem and postmortem bloodstains by estimation of the levels of myoglobin as well as some nucleotides and bases.

MATERIAL AND METHODS

Blood Samples :

- Antemortem (AM) blood samples:

Peripheral venous blood was collected from 40 healthy adult volunteers.

- Postmortem (PM) blood samples:

Cardiac blood was obtained from autopsy cases (40 corpses) with different postmortem intervals and different causes of death. They were referred to Alexandria Forensic Medicine Directorate for postmortem autopsy examination.

- Another AM (n = 10) and PM (n = 10) blood samples were treated similarly and tested blindly to assess the efficacy of the method under investigation.

Preparation of bloodstains and extraction : (Miyaishi et al., 1996).

Two ml of each blood sample were applied on cotton cloth and allowed to soak. The stained cloth then air dried, fixed to cards with the necessary information and stored at room temperature (about 25°c) till analysis. The analysis was done at the following time intervals to study the effect of storage time: one day, 15 days, one month, two months, four months and six months.

At analysis, squares of 1 cm^2 were cut from different stained cloth and immersed in 2ml of 0.01 M phosphate buf-

fered saline, then incubated at 4°c over night. This was followed by centrifugation and the supernatant was used for determination of the followings:

- Myoglobin level by radioimmunoassey (Reese and Uksik, 1981) using myoglobin RIA in vitro diagnostic test kit.

- Adenosine triphosphate (ATP), xanthine and uracil levels by reversed phase high performance liquid chromatography (HPLC) using ultraviolet detector (Nishikawa et al., 1991).

RESULTS AND DISCUSSION

Antemortem blood samples were collected from 40 healthy volunteers (31 males and 9 females). Their ages ranged from 18 to 55 years with a mean of 35.11 ± 4.16 years.

Forty postmortem blood samples were obtained from 29 male and 11 female corpses with a mean age of 36.40 ± 11.12 years (range of 15-63 ys). No significant difference was found between the ages of the two groups (t = 0.687).

As regards the causes of death of autopsy cases, trauma was the cause of death in 28 cases (70%) in the form of stab wounds (n=9), burns (n = 8), head injury (n = 6) and firearm injury (n = 5).

Non traumatic death occurred in 12 cases (30%), 8 of them (20%) were due to pathological conditions: (e.g pneumonia, rheumatic heart disease, pulmonary embolism and intracerebral haemorrhage), while 4 cases (10%) were due to carbon monoxide poisoning.

The postmortem interval of the autopsy cases ranged from 12 hours to 6 days.

Myoglobin Level :

The study showed that, myoglobin level in antemortem bloodstains of the healthy volunteers ranged from 0.06 to 0.13 mg/L with a mean of 0.097 ± 0.02 mg/L (Table 1). Nearly the same results were reported in other studies (Reese and Uksik, 1981; Kagen, 1983 and Mowafi et al., 1993).

On the other hand, myoglobin level in postmortem blood stains ranged from 5 to 54 mg/L with a mean of 32.47 ± 15.76 mg/L (Table 1). This level showed a highly significant increase than the antemortem levels, where t = 12.99 (P = 0.000).

Miyaishi et al. (1996), found that the level of myoglobin starts to elevate immediately after death, and they identified blood as of postmortem origin within 8 hours after death by the level of myoglobin. This elevation could be due to muscle autolysis with release of large amounts of myoglobin (Miyaishi, 1993).

Myoglobin level in postmortem bloodstains showed significant increase with increase of postmortem interval, where F = 100.52, P = 0.000 (Table 2). The lowest level (5mg/L) was recorded in blood taken from a corpse of 35 year old male 12hours after his death from stab wound in the abdomen, and the highest level (54 mg/L) was present in blood taken from a 30 year old female 6 days after her death from carbon monoxide poisoning.

In a study conducted by Puschel et al. (1995), postmortem interval was the most important factor that affects the level of myoglobin. There was an obvious increase in myoglobin level with the passage of time due to progression of autolysis process.

As regards the effect of the cause of death on myoglobin level, the study revealed no statistically significant difference between myoglobin levels in traumatic (32.71 ± 11.16 mg/L) and non traumatic cases (35.48 ± 13.22 mg/L), where t = 0.635, although the highest levels were found among burn and carbon monoxide fatalities. Also by using matched pair technique, the study showed that matched pairs (according to postmortem interval) of traumatic and non traumatic deaths could not be differentiated from one another. This might be due to short survival period of traumatic cases. Takatsu et al. (1991), did not find any correlation between myoglobin concentration and the cause of death.

In the present study, storage of bloodstain samples at room temperature showed no significant change in the level of myoglobin either in antemortem (F = 0.892) or postmortem samples (F = 1.027), which stayed nearly the same along all the storage periods (one day, 15 days, one month, two months, four months and six months). Myoglobin could be detected at high levels in postmortem blood stain samples till 6 months. On the other hand, Miyaishi et al., (1996), could detect myoglobin in a one-year old bloodstain.

ATP, Xanthine and Uracil Levels : (Figure l)

In the present study, the mean level of the nucleotide ATP in antemortem bloodstains was 49.25 ± 9.74 µmol/L (Table 1). Sugie et al. (1995), recorded also the presence of ATP in large amounts in antemortem blood.

The study revealed that during storage there was gradual decrease of ATP levels in antemortem bloodstains with passage of time (F = 13.42, P = 0.000) and still could be detected up to 6 months (Figure 2). On the other hand, ATP could not be detected in postmortem bloodstains and stayed undetectable along all the storage periods i.e up to 6 months.

Xanthine and uracil have not been detected in antemortem bloodstains in the present study and stayed undetectable up to 6 months. This is because ATP is slowly metabolised into ADP and AMP in antemortem bloodstains during storage but hardly metabolised further into its bases (Sugie et al., 1993). While in postmortem bloodstain samples, xanthine and uracil were present with mean levels of 19.51 ± 3.06 µmol/L and 13.37 ± 3.88 µmol/L respectively (Table 1). The same findings were reported by Sugie et al. (1995).

Storage of postmortem bloodstain samples up to 6 months had no significant effect on the levels of xanthine and uracil which remained nearly constant along all the storage periods (Figure 2). This is because the enzymatic and non enzymatic reactions occur very slowly in blood in dried state (King, 1974).

Again, the levels of both xanthine and uracil showed significant increase with increase of postmortem interval, where F = 32.58 and 37.01 respectively (P = 0.000), (Table 2).

The following mechanisms are considered to cause these differences of the nucleotide and its metabolites between antemortem and postmortem blood: ATP in red cells is catabolised along the metabolic pathway (ATP-ADP, AMP-Inosine, hypoxanthine, xanthine). Without a supply of ATP-generating glucose, ATP is exhausted in several hours. Therefore, ATP in corpse blood cells decreases very rapidly with the complementary increase of ADP and the other metabolites. These changes occur so rapidly that ATP cannot be detected in blood only 2 hours after death and the metabolites, inosine, hypoxanthine, xanthine and uracil have increased to significantly high levels (Gardiner et al., 1989 and Sugie et al., 1995). Therefore, a bloodstain containing a significant amount of xanthine and uracil can be regarded as postmortem.

As regards the cause of death and the levels of xanthine and uracil, the study revealed that the levels of both bases were significantly higher in deaths due to pathological conditions and CO poisoning (n= 12) : 22.65 ± 2.31 and 17.53 ± 1.97 µmol/L respectively when compared with the levels in traumatic deaths (n = 28) 16.30 ± 2.57 and 11.21 ± 2.08 µmol/L respectively where t = 7.427 and 9.146 respectively (P = 0.000). This could be due to the presence of antemortem hypoxia in the former conditions (Poulsen et al., 1993 and Gardiner et al., 1990).

Upon doing blind study on 10 AM and 10 PM bloodstains by determination of myoglobin, ATP, xanthine and uracil levels, all the specimens were correctly discriminated, which gives this method 100% sensitivity and specificity. So it is suggest-

ed that determination of these parameters could be applicable in forensic practice as a method for discrimination between antemortem and postmortem bloodstains.

Table (1) : Mean levels of myoglobin, ATP, xanthine and uracil in antemortem (AM) and postmortem (PM) bloodstain samples .

	Antemortem bloodstains (n = 40)	Postmortem bloodstains (n = 40)	t
Myoglobin (mg/L)			
Range	0.06 - 0.13	5 - 54	
X ± SD	0.097 ± 0.02	32.47 ± 15.76	12.99*
ATP (µmol/L)			
Range	35 - 65	---	
X ± SD	49.25 ± 9.74	---	
Xanthine (µmol/L)			
Range	---	15 - 24	
X ± SD	---	19.51 ± 3.06	
Uracil (µmol/L)			
Range	---	8 - 19	
X ± SD	---	13.37 ± 3.88	

* : significant

Table (2) : Effect of postmortem interval (PMI) on the levels of myoglobin, xanthine and uracil in postmortem bloodstains (n = 40) .

PMI	No.	Myoglobin mg/L	Xanthine µmol/L	Uracil µmol/L
12 hr	2	5.50 ± 2.12	8.61 ± 0.71	15.48 ± 0.69
1 day	6	18.33 ± 2.68	9.80 ± 0.83	16.32 ± 1.03
2	10	27.87 ± 2.03	11.67 ± 1.18	18.40 ± 1.17
3	12	34.11 ± 3.31	13.49 ± 1.14	21.11 ± 1.23
4	5	38.91 ± 1.61	15.73 ± 1.58	21.86 ± 0.66
5	2	43.5 ± 1.47	16.55 ± 0.07	22.57 ± 0.70
6 days	3	51.13 ± 3.52	18.64 ± 0.59	23.88 ± 0.91
F		100.52*	32.58*	37.01*
P		0.0000	0.0000	0.0000

* : significant

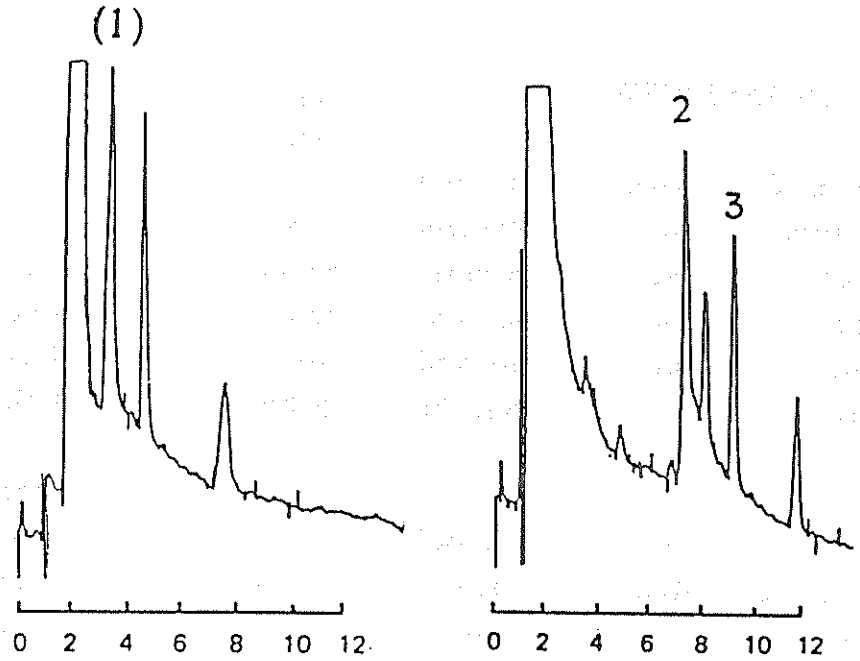

Fig. (1) : Chromatogram of the extract from bloodstains
(A) : antemortem. (B) : postmortem.
Peaks; 1 : ATP. 2 : Xanthine. 3 : Uracil.

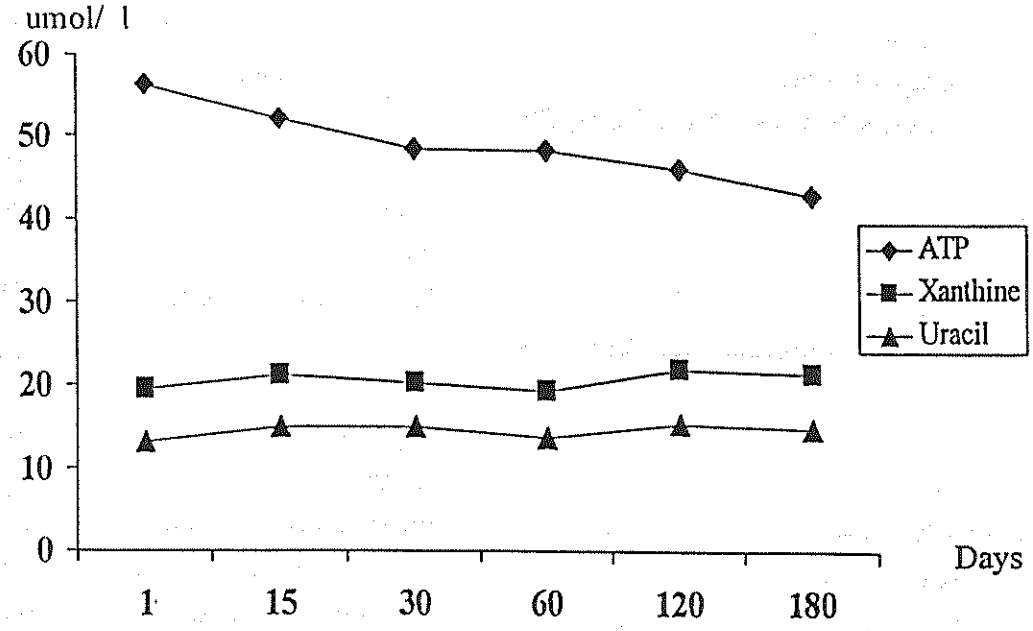

Fig. (2) : Effect of time of storage at room temperature on the level of ATP in AM bloodstains as well as Xanthine and Uracil in PM bloodstains.

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هل هذا الدم قبل أو بعد الوفاة ؟

المشتركون فى البحث

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تعتبر دراسة البقع الدموية من التحاليل المطلوبة فى مجالات عديدة فى الطب الشرعى وبعض حالات التسمم. وقد أجريت هذه الدراسة لإيجاد وسيلة للتمييز بين البقع الدموية من دم قبل الوفاة ودم بعد الوفاة. وكانت الطريقة المستخدمة هى قياس مستوى كل من الميوجلوبين بواسطة القياس المناعى الاشعاعى، وادينوسين ثلاثى الفوسفات، ذانثين واليوراسيل بواسطة جهاز كروماتوجرافيا الضغط العالى .

وقد أظهرت الدراسة وجود إرتفاع ذا دلالة إحصائية عالية فى مستوى الميوجلوبين فى البقع الدموية لدم بعد الوفاة عنه فى البقع الدموية لدم قبل الوفاة. كما لوحظ أيضاً وجود الادينوزين ثلاثى الفوسفات بتركيز مرتفع فى جميع عينات البقع الدموية من دم قبل الوفاة ولم يكتشف وجوده فى أى من البقع الدموية لدم بعد الوفاة .

ومن ناحية أخرى فقد وجد كل من الذانثين واليوراسيل فى جميع عينات البقع الدموية لدم بعد الوفاة ولم يكتشف وجودها فى أى من عينات البقع الدموية لدم قبل الوفاة وقد كانت هذه النتائج فى جميع البقع الدموية بغض النظر عن عمر البقعة - الفترة بعد الوفاة أو سبب الوفاة .

يتضح من هذه الدراسة أنه بتحديد مستوى كل من الميوجلوبين وادينوسين ثلاثى الفوسفات، الذانثين واليوراسيل فى البقع الدموية الجافة يمكن التمييز بين دم قبل الوفاة وبعد الوفاة فى مجال الطب الشرعى .