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Original communication

# Evaluation of procalcitonin postmortem levels in some models of death: An experimental study

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ABSTRACT

Post-mortem determination of biochemical parameters, especially for obscure cases, has been recognized useful in diagnosis of the underlying causes of death. Procalcitonin (PCT) is known to rise in a response to any proinflammatory stimulus. The present study aims to estimate postmortem PCT levels in serum and kidney, liver, brain; and whether it is similar in different causes of death models (trauma, drowning and freezing) models or not. The study was performed on 60 male rabbits. Rabbits were divided into four different death induced models (15 rabbit each): trauma, infection, drowning and freezing models. At the end of the study, all rabbits were sacrificed; blood samples, kidneys, livers and brains were collected. PCT was measured using ELISA assay. Results showed highly significant increase in PCT levels in all tested samples in different models of death. The infection induced model showed the highest levels in all tested samples compared to other groups mainly in liver; followed by trauma model and drowning model which were increased mainly in brain's samples. The least model which showed increased PCT levels was the freezing model mainly in liver samples. Post Hoc multiple comparisons test showed significant differences between groups in most of liver, brain and kidney samples, while PCT serum blood samples were significant only between trauma and infection groups. It was concluded that PCT can differentiate between sepsis and non-sepsis related deaths and that organs like liver, kidney and brain PCT levels could be an alternative to serum PCT for the diagnosis of postmortem sepsis.



## 1. Introduction

In practical casework, the pathologist is often confronted with Systemic Inflammation Response Syndrome (SIRS) e.g in trauma, hypothermia) and sepsis as possible causes of death. However, autopsy findings and histological imaging are often unspecific and therefore not conclusive for their diagnosis.[1]

Postmortem biochemistry and molecular biology were used importantly to investigate the systemic pathophysiological changes involved in the process of death that cannot be detected by morphological methods and these may be called pathophysiological vital reactions.[2,3]

Procalcitonin (PCT) is the precursor of the calcitonin hormone, which is responsible for calcium homeostasis and it is a peptide that composed of 116 amino acids. It is produced by parafollicular cells of the thyroid gland and by the neuroendocrine cells of the lung and intestine. The blood level of procalcitonin rises in a response to any pro-inflammatory stimulus, to be above the limit of detection (10 pg/mL) in clinical assays in healthy individual. It rise with infection especially bacterial origin while viral or non-infectious inflammations does not raise it significantly. The blood levels of procalcitonin may rise to a level 100 μg/L with severe systemic infection.[4]

Systemic inflammation and immune responses are involved in deaths due to trauma and disease.[5] In forensic practice, one of the essential roles of legal medicine is to provide a different approaches to difficulties that may face medicine and law as a forensic pathologist may face a lot of limitations regarding the precise diagnosis of septic or traumatic deaths.[6]

Postmortem diagnosis of sepsis may be difficult due to non-specificity of macroscopic and routine histological findings; in addition, when clinical data on a deceased previous medical history are not available during medico-legal autopsy.[7] Meanwhile, traumatic deaths could happen without notable fatal injuries to organs or soft tissues as well as there may be a difficulty to determine traumatic injuries that were developed whilst the victim was alive.[8]

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Postmortem biochemistry of body fluids and tissues may provide an understanding of the death process and its systemic pathophysiological changes that cannot be discovered by the ordinary morphological methods.[3]

The present study aims to estimate the postmortem levels of PCT rise in the serum and some organs (kidney, liver, brain) of animal induced septic death model; and whether such rise is similar to other causes of death models such as traumatic, drowning and freezing death models or not.

## 2. Material & methods

### 2.1. Study design

The experimental study was performed on 60 male rabbits, their weights ranged from 1.8 to 2.0 kg. They were reared in clean capacious metal cages under suitable laboratory conditions (as good aeration, lightening and a constant room temperature about 24 °C). The animals allowed to have free access to water and standard rabbit fodder (25.1% proteins, 3.8% fat, 18.05% cellulose); at Animal House of Medical Experimental Research Center, Faculty of Medicine, Mansoura University.

According to Egyptian/European Union legislation (86/609/EEC), the experimental animals were housed, handled, and euthanized. The Ethical Committee of the Mansoura Faculty of Medicine approved the present experimental protocols.

#### 2.1.1. Animals were divided into four groups

1 Group 1 (traumatic induced death model):

Fifteen rabbits were assigned as a trauma model induced death.

2 Group 2 (infection induced death model):

Fifteen rabbits were assigned as a septic model induced death.

3 Group 3 (drowning induced death model):

Fifteen rabbits were assigned as a drowning model induced death.

4 Group 4 (freezing induced death model):

Fifteen rabbits were assigned as a trauma model induced death.

At the end of the study, all rabbits were sacrificed by decapitation. Postmortem blood samples were collected (2 ml) directly after death of each rabbit. Each blood sample was centrifuged immediately and sera were stored at −20 °C.

Kidneys, livers and brains were collected from all animals and rapidly frozen at −20 °C.

Procalcitonin was measured using enzyme linked-immunosorbent assay Kit in serum and organs (kidney, liver and brain) samples. Cat No. 201-12-0978 Sun Red Bio-technology Company. All analyses were performed in accordance with instructions by supplier. The optical density will be measured under 450 nm wave length with assay range: 6–2000 pg/ml.

### 2.2. Methods

1 Induction of traumatic death model[9]:

The anaesthetized rabbit has to be secured in a stereotactic frame. A round steel disc (1 cm diameter, 3 mm thickness) is fixed by surgical cement onto the central area of the skull. A 450 g weight is dropped from a height of 1 m onto the center of the steel disc.

2 Induction of Infection death model[10]:

Rabbits exposed to an endotoxic shock after they were received an intravenous solution of 200 μl lipopolysaccaride (LPS) at different concentrations.

3 Induction of drowning death model:

It was done by immersing the animal cage in a basin filled with water till the animal was died.

4 Induction of freezing death model:

It was done by exposing the animals to a (−20 °C) environment till death.

#### 2.2.1. Statistical analysis

The data were analyzed by using SPSS (statistical package for social science) program version 16. The data were expressed as mean and standard deviation. On way ANOVA (F test) was done to compare between the studied groups. Post Hoc benferroni test was done to study the difference between each two groups. Significance was considered at p value less than 0.05.

## 3. Results

The levels of procalcitonin (PCT) in all tested samples of different causes of deaths are highly significantly increased as shown in Table 1 and Fig. 1.

In trauma induced model, PCT levels obtained from liver samples were the highest among other organs in the same group followed by the kidney's levels (Fig. 1).

Meanwhile, in infection induced model, the PCT levels were highly elevated in all tested samples in comparison to the other groups. The levels in the liver samples were the highest compared to other organs in the same group followed by the brain's levels (Fig. 1).

In drowning induced model, the PCT in the brain samples showed the highest elevated levels compared to other organs in the same group followed by the kidney's levels (Fig. 1).

In freezing induced model, the PCT levels showed increase in all tested samples but were less than other groups. The levels in the liver samples were the most elevated compared to other organs followed by the brain's levels (Fig. 1).

**Table 1**
Procalcitonin levels (pg/ml) in serum, kidney, liver and brain samples of different models of death in rabbits (n = 60).

| Death model | Serum (mean ± SD) | Kidney (mean ± SD) | Liver (mean ± SD) | Brain (mean ± SD) |
|---|---|---|---|---|
| Trauma | 593.12 ± 240.6 | 917.34 ± 80.77 | 1309.58 ± 105.24 | 672.62 ± 46.33 |
| Infection | 1684.87 ± 873.18 | 1831.73 ± 26.15 | 2103.8 ± 107.67 | 1922.32 ± 83.45 |
| Drowning | 589.35 ± 326.91 | 861.43 ± 120.35 | 809.44 ± 72.44 | 1294.46 ± 202.94 |
| Freezing | 194.09 ± 45.43 | 189.31 ± 16.92 | 651.72 ± 214.02 | 358.16 ± 109.76 |