INTRODUCTION

Estimation of time since death is one of the primary objectives of an autopsy. Forensic Scientists and researchers have been working hard to find out methods of accurate determination of postmortem interval since time. However, the concept of "Postmortem Clocking" so far seems to be a distant dream only. The favorite biological fluids, to study postmortem biochemical changes, have been those which withstand putrefactive changes for longer duration, like vitreous humor, cerebrospinal fluid, pericardial fluid **(Garg et al., 2005).**

Autopsy surgeons continue to rely on age old subjective methods of observing the degree and chronological staging of external as well as visceral postmortem somatic changes like cooling of the body, rigor mortis, changes in the eyes, hypostasis, signs of decomposition, mummification, adipocere formation, maggot infestation , all these methods are corroborated with circumstantial evidences for PMI detection.**(Gautam, 2010).**

In blood, markers like electrolytes, urea, creatinine, glucose have been more commonly studied. Enthusiastic studies have been undertaken by various researchers to find out reasonably reliable methods of estimating postmortem

interval by studying serial quantitative changes in serum levels of various enzymes and to interpret the data obtained therefore in terms of duration of death. However, the accuracy of such an opinion remains big area of concern even today, as the range of duration is mostly too wide to be practically useful. **(Garg et al.,2005).**

AIM OF THE WORK

The aim of the present study is:

- To investigate the potential use of serum (cTnI) as an estimator of postmortem interval via application of certain quantitative methods in different periods of time and different degrees of temperature .
- To consolidate the validity of this marker by comparing its results versus postmortem histo-pathological changes in the rat's heart tissues.

Chapter (I)
Methods of Estimation of Post Mortem Interval (PMI)

Precise estimation of the postmortem interval is a difficulty accompanying forensic medicine from its very beginning. Following a preliminary diagnosis of the cause of death and with the help of investigative techniques, the pathologist supports the prosecutor's office and the police by leading investigations to the appropriate direction**. (Knight, 2002).**

From the point of view of criminal law, a precise estimation of the time of death (TOD) enables to verify witnesses' statements, limit the number of suspects and assess their alibies. **(DiMaio and DiMaio ,2001).**

Also determination of the TOD sometimes gains relevance regarding civil law, since it may have impact on the order of inheritance or possible commitments resulting from the order of deaths. Such problems may occur if corpses of two or more related persons are found simultaneously e.g. in case of multiple homicide or accident with several deceased**. (Knight and Saukko, 2004).**

Despite attempts over a hundred years to develop methods for precise estimation of the TOD, the accuracy of these methods, both when using one of them or combining

several of them, still leaves a margin for improvement. During the first 6 h after death, there is at least a 2 h margin of error, but in the next 14 h, the margin of error increases to at least 3 h, and during the following 10 h this margin of error increases to approximately 4.5 h. The precision of the conventional methods decreases significantly with time. Therefore, usually since about 24 h postmortem all estimations are rather rough approximations. **(Knight, 2002).**

Currently known methods of PMI estimation can be divided into three groups according to Kaliszan et al., 2009:

1. The first group comprises methods based on analysis of the process of cooling of corpses and the body temperature measurement i.e. temperature-based methods. Methods based on the body cooling process are considered to be more precise when used in the early post mortem period (first 24 h).
2. The second group consists of methods based on assessment of post mortem processes occurring in corpses. Some of them especially those dealing with the early changes, can be applied when the time since death is counted in hours while others which are based on late changes, can be used if the post mortem interval is counted in months or years.

3. The third group consists of methods combining body temperature measurements and observation of postmortem changes**.**

1. Methods based on the body cooling process (algor mortis):

Postmortem cooling of the human body is the loss of heat to the surroundings until the internal temperature reaches that of the environment, this takes place by three major mechanisms:

a. **Conduction:** transfer of heat by direct contact to another object specially in internal organs.

b. **Radiation:** transfer of heat to the surrounding air by infrared rays.

c. **Convection:** transfer of heat through moving air currents to the body **(Joshua, 2006).**

In **1863, Taylor and Wilkes** reported on measurements of body temperature using a clinical thermometer applied to the skin of the abdomen. But this method of examination does not provide an acceptable reliability of results due to the influence of external factors and the manner of applying the thermometer.

In **1868**, Professor **Rainy,** being the first to apply Newton's law of cooling to the process of cooling of a corpse, considered the ambient temperature as an influencing

factor. By repeatedly measuring temperatures in the rectum of deceased individuals he managed to establish experimentally the gradient of the curve of temperature decrease versus time, according to Newton's cooling coefficient. Moreover, he confirmed the existence of an initial phase of slower temperature decrease, later described by Shapiro as plateau phase. **(Shapiro, 1965)**

In **1880, Burman** measured temperatures within armpit using a self-made thermometer, allowing to read temperatures without removing the probe from an armpit, he concluded that the decrease of temperature in the first 12 h post mortem is linear and equivalent to approximately 0.9 °C/h.

Following an interruption of research on methods for the estimation of PMI, most likely due to World War II (WWII), **(Schwarz and Heidenwolf, 1953)** presented the first standardized sigmoidal curve, showing a significant slowdown in cooling of a corpse in the initial period post mortem. This curve was supposed to be applicable within a limited temperature range (around 17 °C) for any body weight and any clothes on the deceased. However, Schwarz and Heidenwol's curve never gained practical use**.**

In **1956, Lyle and Cleveland** used a 6-channel thermometer allowing continuous recording of temperatures in six different locations: chest and forehead skin, rectum,

liver, brain and thigh muscles. Although, they did not develop any useful methods for determination of the PMI, they observed some interesting facts. It turned out that temperature–time curves for the brain are the most regular, and such curves for skin are the least regular ones. They also pointed out that time elapsed since death cannot reliably be determined after 24 h from the moment of death or when body temperature decrease approaches the ambient temperature.

Henssge and coworkers ,2000 presented a simplified method to calculate Newton's cooling coefficient and determined statistical values of deviation between calculated and actual PMI for the process of cooling under standardized conditions. His formula was based on examination of the process of body cooling of numerous corpses and also using special phantoms which are dolls filled with gel to simulate a human body.

Researchers broadened the range of applications of the method to include various conditions of cooling. They also introduced empirical corrective multipliers for body mass, various types of clothing and conditions at the scene of death. As a result they created nomograms, used as a standard method by forensic medicine specialists, which allow to read instead of calculating the TOD , as shown in **Figure (1).**

Computer software created from this research is also helpful and used for PMI estimations**. (Henssge and Madea, 2004).**

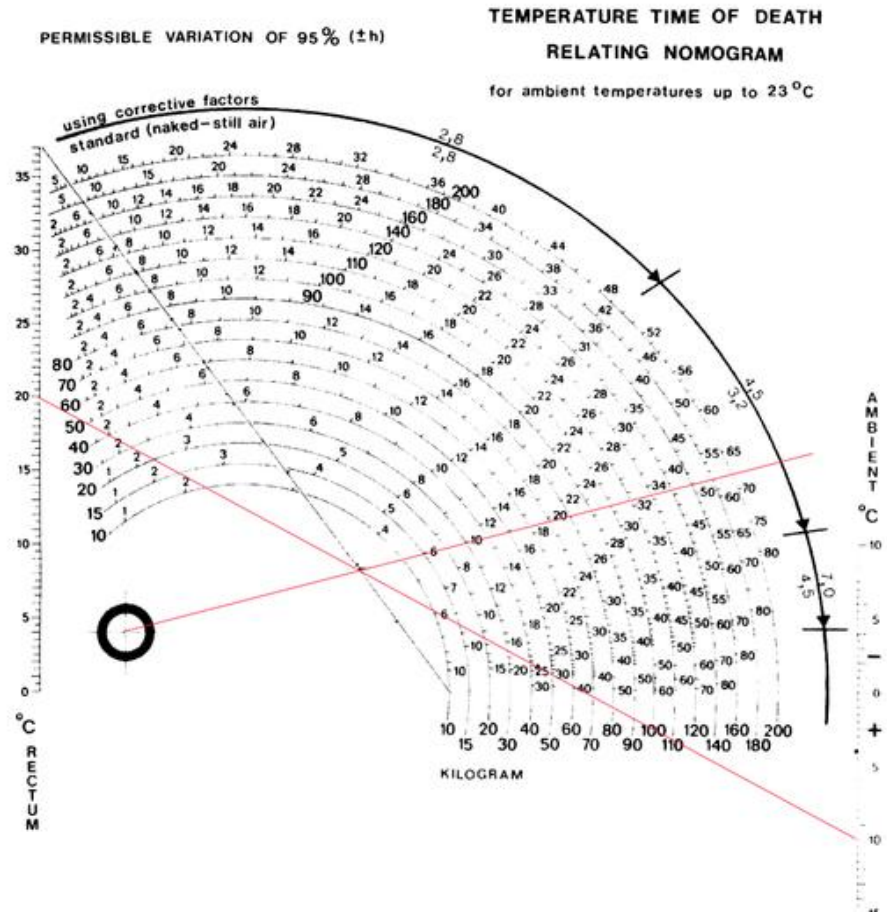

Figure (1): Henssge nomogram for calculation of time of death based on body temperature measurements **(Henssge et al., 1984).**

Attempts were also taken to use other body sites to record body temperature. The brain turned out to be a possibility for relatively precise assessment of PMI,

especially in the first 6 h post mortem (accurate to ±1.5 h). **Henssge, et al., 1984** developed a nomogram for the brain which enabled easy reading of time passed since death using the same standards as for temperatures in the rectum.

An advantageous factor regarding the brain is the lack of influence by the body mass on its temperature. However, the error of PMI estimation may increase e.g. because of varying amounts of hair or covering of head (i.e. cap, hat) influencing the thermal isolation of the brain. A factor contributing to making the use of brain temperature measurements more difficult is the necessity to insert a probe inside the skull. However, current technical development also allows to record brain temperature by non-invasive methods such as magnetic resonance spectroscopy **(Karaszewski et al., 2006).**

In 1986, Miścicka-Śliwka and Śliwka published a series of papers, describing own examinations on the usefulness of corpse temperature measurements at several body sites to assess the PMI. It was found that the heart might be a reliable site for temperature measurements. They developed a nomogram enabling reading the PMI based on heart temperature measurement, taking into account the circumference of the chest. **(Karaszwski et al.,2006).**

In **1996, Baccino et al**. performed a series of experiments based on outer ear temperature measurement.

Temperatures were recorded by a probe inserted into the ear, its tip with contact to the tympanic membrane. A plateau of post mortem decrease was not observed in outer ear temperature. The method was considered useful if ambient temperatures are between 16 °C and 23 °C. According to the authors, tympanic temperature closely reflects the cerebral temperature and the results were therefore characterised by accuracy similar to that of the nomogram method by **(Henssge et al., 1984)**. Besides, in the initial period after death the accuracy was even better. Additionally, an important advantage was that it represents an alternative method when suspecting a sexual background of a crime i.e no infringement of the rectum.

Al-Alousi et al., **2001** developed a method based on microwave thermography in three body sites: brain, liver and rectum. They calculated curves of the average course of cooling within certain body regions, separately for naked and covered bodies. However, this method requires basic knowledge of organ temperatures at the moment of death, and inaccuracy in this matter may result in wide errors in PMI estimation.

In **2005, Kaliszan et al**., reported about temperature measurements in the eyeball and soft tissues of the orbit as of possible usefulness in determination of the post mortem interval. Precision of PMI estimation based on temperatures of the orbit soft tissues is more reliable than

that of muscles and of rectum up to 10 h after death. However, **Honjyo et al., 2005** suggested that better assessment is ensured by data obtained from temperature measurements in muscles and in the rectum in the later post mortem interval**.**

2-Methods based on assessment of other post mortem changes:

Among methods for estimation of the PMI, those based on postmortem changes in the body during the first few days following death, the following appear to be noteworthy: drying out of the cornea, assessment of intensity and mobility of hypostasis (lividity) and development and receding of rigor mortis **(Knight, 2002).**

Ocular changes include a thin film appears over the cornea of opened eye within minutes of death and in closed eyes within hours, followed by corneal cloudiness 2-3 hours in open eyes and 24 hours in closed eyes, Tache' noire-(blackish discoloration) develops, then no intraocular fluid after four days **(Liu et al., 2008).**

Hypostasis, Lividity, Livor Mortis, Darkening of Death:

Burton in **1974,** gave to hypostasis the dramatic name of "the darkening of death". **In 1995, Pounder** explained that the bluish tinge to this blood is not due to a pathological process such as cyanosis but due to continued oxygen

disassociation and a reflux of deoxygenated venous blood into the blood vessels. Interestingly, under low ambient temperature, lividity can adopt a bright pink colouration due to resaturation of haemoglobin with oxygen.

Pless et al., 1997 described the process of hypostasis that it occurs when circulation stops and gravity acts on the stagnant blood bringing it down to the lowest levels of the body. This process of settling is able to occur because, within 30-60 minutes, the blood becomes in coagulable due to the release of fibrinolysins, and thus remains in a liquid form.

Lividity can present within 20-30 minutes postmortem, first as small patches which will coalesce over time and become fixed after 10-12 hours **(Pounder, 1995).** In **2004, Amendt et al.,** Stated that lividity is generally evident after two hours and is fixed after four-six hours after death due to the fat in the dermis solidifying in the capillaries.

Knight, 2004 referred hypostasis to as "lucidity, "staining" or "cogitation"**.** He also discussed that the process of hypostasis has no relevance to the PMI as its onset is variable, and it may not be visible in infants, the senile or anaemic persons.

Rigor Mortis, Stiffening of Death:

Burton, 1974 explained "The stiffening of death" is a well known process of decomposition is rigor mortis. **Pless et al., 1997** stated that the process is initially apparent in the eyelids, jaw and neck and progresses down the body with the arms, trunk and then legs being affected. The whole body is in a rigor state at approximately 12-24 hours postmortem**.**

In **1997, Gill-King** explained that the sarcoplasmic reticulum of muscle cells begins to disorganise and calcium ions flood into the sarcomere, which consists of alternating parallel protein filaments of actin and myosin. The calcium ions unblock the binding site of the actin molecule allowing the two molecules to bind via a cross arm (also known as a cross bridge) extending from the myosin as shown in **Figure (2) .**When this cross arm is pulled back the actin molecule is pulled along the myosin, consequently the overall muscle tissue is shortened and becomes firm and rigid. As the Adenosine triphosphate (ATP) levels in the body have decreased; this link between the actin and myosin cannot be chemically reversed, thus rigor is induced and can last as long as 84 hours.

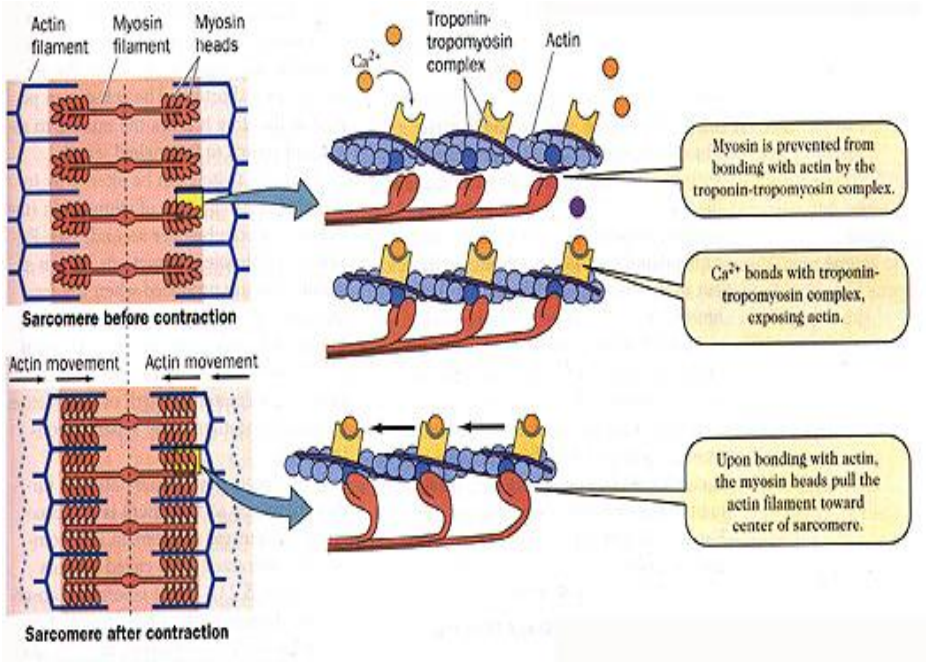

Figure (2): Muscle contraction mechanism.**(Phsioweb.org, 2011).**

Rigor desists after a passage of time. This is believed to be caused by an occurrence of a structural change in the myofibrils. It is believed that proteolytic enzymes may be involved in the detachment of actin and myosin **(Gill-King, 1997).** According to **Jackson and Jackson, 2004,** Rigor mortis is evident in about 2-6 hours following death **a**nd it is not permanent with a post rigor flaccid state occurring at approximately 24-36 hours postmortem.

The process of flaccidity begins in the same sequence as rigor began (eyes, jaw, neck etc.). Once rigor is evident it is possible to "break" it by manual manipulation. Rigor can in