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Emergence of biochemical methods for estimation of post-mortem interval – A review

Ankita Kiritbhai Parmar
Department of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Lavad-Dahegam, Gandhinagar, Gujarat, India

Abstract
Post-mortem interval (PMI) is the most important part of every medico-legal investigation as it narrow down the field of investigation. In the past decade many methods have been introduced for the precise estimation of PMI. The estimation of PMI should be highly accurate as the whole investigation depends on it. The traditional methods which were used in the beginning of the decade were bound in the time limit. Such methods like rigor mortis, livor mortis, algor mortis, decomposition etc can only provide some superficial idea about the PMI in the early death hours. But with the rising era of biochemical methods the whole face of investigation has changed and the medico-legal investigation has attained the new levels of accuracy which were never before. But all these methods now need a restart to become more rapid, sensitive and cost effective. Here the review describes the emergence of biochemical methods as an efficient tool for the determination of PMI.

Keywords: Post-mortem interval, medico legal investigation, rigor mortis, algor mortis, livor mortis, decomposition, bio chemical methods

Introduction

During the last decade the estimation of post-mortem interval (PMI) has become most extensive, tedious, and collaborative subject. Post-mortem interval is defined as the interval between the death and the time of post-mortem examination. It is the most vital part of medico-legal investigation as it may lead to the correct attribution of the circumstances of death [1]. It provides most essential part of information which can be used in investigating the crime and other unattended deaths. It provides a clue to set up proper enquiries to detain the culprit and to abolish the naïve. Precise determination of post-mortem interval gains relevance regarding the civil laws and the criminal laws where accuracy in determining the time since death may prohibit or embrace the suspect or accused from a scrupulous homicide. Even though a large number of efforts have been employed to widen techniques for accurate determination of postmortem interval, it still leaves a fringe for enhancement. This is due to the changes in body found after death, are frequently ruled by capricious factors [2, 3, 4].

Till the date a large number of methods have been used to determine the postmortem interval, these include study of physical, chemical, biochemical, histological and enzymatic changes which occur progressively in a dead body [4]. Due to its great significance in medico-legal investigations, as an outcome more than a few works have been reported to offer the possibilities of truthful prophecy of post-mortem interval [5, 6, 7].

After the death the body undergoes so many changes i.e.,

- Cooling of the body (Algor Mortis),
- Rigor Mortis or Cadaveric Rigidity,
- Metabolic Processes (supravital reactions),
- Livor mortis,
- Putrefaction,
- Biochemical changes in different body fluids, etc.

The study of such changes with respect of time helps to determine accurate post-mortem intervals. For convenience methods for determination of post-mortem interval can be divided in to two main parts,

1 Traditional or physical methods
2 Biochemical methods.

Traditional methods include algor mortis, rigor mortis, livor mortis, and putrefaction; while biochemical changes include changes in various body fluids and tissues. Physical methods are the main basis to determine the post-mortem interval. It takes place in early post-mortem intervals and come in existence due to the effect of external factors. Although traditional methods are methods which are in use for several past years, they are likely to produce invalid results. In spite of that biochemical methods are quick, simple, reliable, straight forward and modern. Such methods give more accurate consequences with negligible slip-ups. Still both methods have their own undoubting magnitude in determination of time since death [8].

Here the purpose of the present study is not to reiterate the known facts on post-mortem interval but to hark back some uncertain common tribulations pertaining to PMI which should be remembered in practical casework and in future studies.

This review points out the methods that are used to determine post-mortem interval. Traditional methods would be mentioned briefly to throw light on the emergence of biochemical methods which definitely is having an edge over the past methods.

Traditional methods

1. Cooling of the dead body (Algor mortis):

The gradual decrease in body temperature is one of the most prominent early signs of death. The amount of cooling signifies the approximate time since death during the first 24 h after death. The fall of temperature of the cadaver occurs due to the facts, that after death there is no heat production, due to loss of all physical, chemical and metabolic functions of the body and there is invariable loss of body heat until it

comes to the level of the environmental temperature, as the heat regulating center is stationary [5, 6, 8].

Harry rainy [9] was the first person who applied Newton's law of cooling to the process of cooling of a dead body. He had repeatedly measured rectum's temperature and established the gradient of the curve of temperature decrease versus time. He also observed that at initial level temperature decreases slowly which was later described as Plateau phase by Shapiro [10]. After Rainy [9] many experiments based on body cooling has been carried out for the same purpose. Henssge *et al*. [14] proposed a nomogram for the brain. They found the temperature of brain to be easier for estimation of time since death. Baccino E *et al*. and Michal K worked on outer ear and eyeball temperature respectively for the same purpose [44, 45]. Likewise, a lot of efforts have been put out to measure precise time since death by measuring temperatures of different body sites and different concepts [11, 12, 13].

2. Rigor Mortis

Rigor mortis is a symptom of death. During the early stage after death, it gives superior suggestion about the time of death. After death rapid muscular relaxation is followed by slow rigidity of the muscles. In short, rigor mortis is that state of the muscles of a dead body when they become stiff or rigid with some degree of shortening. The primary phase of relaxation of the muscles continues for an hour or more after death. The muscles of the body slowly become rigid after passing this period. Such stiffness of muscles indicates the molecular death of that particular muscle [6, 7, 8].

In 1811 Pierre-hubert Nysten provided a scientific description of rigor mortis. He defines the progressive states of cadaveric rigidity during rigor mortis which is known as "Nysten's law". According to Nysten's law rigor mortis first affects the muscles of mastication, then affects those of the face and neck, then those of the trunk and arms and lastly affects the muscles of the legs and feet. Many works have been made to estimate accurate post-mortem interval from rigor mortis [15, 16, 17]. But almost every work suggests that factors like age, body muscular mass, temperature, climate conditions, etc… significantly affects the rate of rigor mortis, showing that rigor mortis is not an appropriate method for the accurate determination of time since death.

3. Livor Mortis (Post-mortem Lividity)

Livor mortis begins very soon after death as it is a function of cardiac activity. It is an early change of the body after death. Post-mortem lividity or Livor mortis is bluish-purple or reddish-purple discoloration due to capillo-venous distention with blood at the undersurface of skin of the dependent parts of the body for settling of blood in those areas due to the fact that blood in motion ceases. In short, it is the discoloration that occurs due to settling of blood after death [6].

By the end of the first hour after death, when the position of the body is undisturbed, the staining will start appearing in form of small patches at the dependent area of the body. Post-mortem staining is a function of cardiac activity. As cardiac activity stops, due to the hydrostatic pressure of the blood, it starts settling down to the lowest points within the body depending on its position, so that area of the body will show discoloration. The pressure of external objects like, clothing affects the formation of post-mortem staining. The completion of spreading of PM staining takes about 5 to 6 h. After formation it gets fixed over the areas. After this fixation, if the position of the body is disturbed; then also the staining will remain as it is [6, 8].

But, lividity will not appear if the body is constantly altering its position i.e.; in case of drowning or the skin of the dead person is dark or in case where much blood is lost.

So post-mortem staining gives an idea about the time of death, but it depends on the cardiac activity which is affected by external factors and on the position of the body. Also, it is found absent in cases like drowning, massive hemorrhages, and so on. So, this method is not appropriate for the accurate determination of post-mortem interval.

4. Putrefaction

After death the protective function of the body is lost. So physical and chemical factors present in the environment, which were not allowed in a live body will start acting on the dead body. The gross structure of the body is maintained up to a certain level while the other processes take place in the body. But as the time increases, the process of wear and tear will start. This is simply known as Decomposition or Putrefaction [6, 8].

Decomposition can be defined as a process by which the complex organic body tissues break down to simpler inorganic compounds, due to autolysis. The process leads to discoloration of the dead body, evolution of foul-smelling gas, swelling of the dead body with gradual and total destruction of the different body parts. The decomposition of different organs of the body occurs on different time interval.

Decomposition is not an early sign of death but it is the definite sign of death. The external factors like temperature, moisture, air, clothing, etc… affects decomposition. As time factor plays an important role in decomposition of body; this method is useful in late post-mortem intervals. Entomological studies have been introduced on the basis of decomposition for the estimation of time since death.

All these traditional methods give a rough idea about time of death, but such methods are not accurate enough for determination of time since death. With the passage of time biochemical methods have replaced the traditional methods, as biochemical methods give more accurate result. Also, these methods are simple, rapid and more precise.

Biochemical methods

Various biochemical methods have been employed since last few years for the estimation of post-mortem interval. Different body fluids like blood, cerebrospinal fluid (CSF), vitreous humor, etc… show changes rapidly or in a short time after death. Such changes in body fluids steps forward in a fair array with time until the body collapse. Each change takes place on a particular time. So, from these changes estimation of post-mortem interval can be done more precisely. Another advantage of such biochemical method is that the environmental factors affect less on body fluids. So, study of changes in body fluid after death can be useful to estimate the precise time since death in comparison with other physical methods.

Fekete & Kerenyi [18] analyzed 160 cases for detection of blood sugar and blood urea nitrogen after death. They reported that there is no correlation between the level of post-mortem blood sugar and the time elapsed since death. They observed that the post-mortem blood sugar did not rise significantly in the last 12 hours before death. They reported

that the post-mortem blood sugar level is governed by several factors like glycolysis by enzymes, temperature of the body, etc… So, they concluded that the blood sugar is unsuitable for the estimation of post-mortem interval and no post-mortem normal of this substance can be established. For blood urea nitrogen they reported that unknown external factors play a part in the rise of post-mortem blood urea nitrogen. They stated that blood urea nitrogen remains constant at the site of collection.

Jenkins [19] worked on 38 autopsy cases for the determination of urea level in post-mortem blood. He concluded that the post-mortem blood urea level is less reliable and shows similarity with ante-mortem blood levels.

Coe [20] demonstrated that urea is the most stable substance in post-mortem blood. He observed that most of all cases majority shows 2 mg/dl variations in the specimens obtained from the same body over 100 h after death. He also found that sodium level decreased rapidly after death and the average rate of fall is 0.9 meq/L per hour. He analyzed a large series of samples for chloride level and found the least square regression rate of fall to be 0.95 meq/L.

Jetter [21] & Schleyer [22] described that plasma chloride decrease with the increase in post-mortem interval. Jetter [21] states about the calcium levels remaining constant after death.

Sawyer *et al* [23] studied the post-mortem changes in the pH of the blood and selected tissues in rat. They noted that cardiac blood pH was significantly decreased in rat blood after death, but no correlation was found between human blood pH and PMI.

Dalbir S *et al*. [24] studied the relationship between serum sodium, potassium concentration and time since death in North West region of India. They conducted the study on 474 subjects. The concentration of electrolytes was measured by Flame photometry and statistical evaluation of the data was done. In this study double logarithmic model of Querido was used to establish a relationship between the time since death and sodium/potassium ratio. They conclude that considerable relationship between PMI and serum sodium, potassium concentration does exist. A rapid increase in potassium concentration was found in first 36 h of death, while sodium decreases with the increase in PMI. They stated that with a mean value of 10.59 ± 3.96 to 6.81 ± 3.27 of ratio of sodium / potassium concentration shows a rapid decrease in first 12 h of death and this decrease is continued to 48 to 58 h of death with a mean value of 2.95 ± 0.13.

T. Sun *et al*. [43] studied the relationship between ATP changes of rabbit blood and PMI. They found that the changes in ATP levels in blood shows relatively stable and regular degradation within 72 h after death at different temperature.

Sabucedo & Furton [25] had worked on Cardiac Troponin-I (cTnI). They used cardiac tissue. The samples were collected within 15 h after death. They demonstrated that post-mortem degradation of cTnI may serve as an estimator of postmortem interval using qualitative comparisons.

Martin *et al* [26] did a pilot study of quantification of mRNA degradation for the estimation of time since death. 10 ante-mortem and 50 post-mortem cases were studied in this study. RNA isolation was performed and the concentration was measured with UV spectroscopy. PCR is done for DNA sequences and the amount of amplification was estimated by ultra thin polyacrylamide gel electrophoresis. They concluded that RNA amounts in both ante-mortem and post-mortem cases failed to show significant relation with PMI. Although RNA degradation can be useful to determine PMI in late hours as it is a slow process.

Till the date large number of studies has been made on Vitreous humor of the eye for the estimation of time since death. Extensive numbers of studies have been made on potassium level of vitreous humor. This is because vitreous humor is protected in eye and less connected with external factors. So the important research made for the determination of time since death using vitreous humor is as follows.

Madea & Henssage [30] were first to conclude that there is a significant rise in the concentration of potassium in vitreous humor with increasing post-mortem interval. They had derived a formula PMI (hours) = 5.26 x K concentration in meq/L – 30.9 which gives accurate result. R. Ahi & V. Garg [27] has measured the potassium concentration in vitreous humor for the determination of post-mortem interval. They have collected vitreous humor samples from 176 bodies. Temperature and humidity level was noted on the time of each collection. Each sample was pretreated and then analyzed in flame photometry. The observations were statistically analyzed and a graph showing the correlation between time since death and vitreous potassium concentration was plotted. They observed a significant increase in the potassium concentration with increasing post-mortem interval. They have also concluded that humidity or temperature do not affect the findings. B K Prasad, A Choudhary & J N Sinha [31] also worked on correlation between vitreous potassium level and post-mortem interval. They collected vitreous humor's sample from 150 autopsy cases. The analysis was carried out using flame photometer. They stated that there is a linear increase in potassium level with respect to increasing time after death.

Yogiraj & Indumati V. [28] collected 100 samples from autopsy cases. They have estimated sodium, potassium and chloride from vitreous humor for the determination of post-mortem interval. They used flame photometer for the analysis of potassium and sodium, while analysis of chloride was done by modified colorimetric method of Schoenfeld and Lewellen. They stated that levels of sodium and chloride remained constant with increase in post-mortem interval. They noted that potassium does increase at an average rate of 0.17 mmol/L. They concluded that potassium is a useful marker for estimation of time since death.

R. van den oever [29] worked on ammonium concentrations in vitreous humor for TSD estimation. He stated that ammonia concentration of vitreous humor do change after death.

R Deokar, A Shendarkar & S Patil [32] worked on calcium levels of vitreous humor. They collected 152 samples in different age group of 7 years to 80 years. The samples were analyzed by automated analyzer. The observations were taken up to 40 h after death. The data was statistically analyzed. They noted that there was a fair increase in calcium level with increasing post-mortem interval. From the statistical data they plotted a graph of calcium concentration against PMI and from the plot they derived a formula Estimated PMI = 1.065(calcium) - 10.8064. They concluded that there is a considerable statistical correlation between calcium concentration and post-mortem interval

and it can be adjunct to vitreous potassium for estimation of time since death with reduced error.

Chandrakant H *et al*. [46] worked on changes in concentration of sodium, potassium and chloride of vitreous humor for estimation of time since death. They collected the samples from both eyes from 114 dead bodies. The concentration of electrolytes was measured by Roche 9180 Electrolyte Analyzer and statistical analysis of the data was done. They stated that sodium and chloride levels in left eye and potassium level in the right eye were higher. They concluded that there is no significant relation between the electrolytes concentration and age and sex of the individuals. They also stated that sodium and chloride concentration in vitreous humor shows a trivial fall with increasing PMI, while potassium shows a minor rise with increase in PMI. So, they concluded that the vitreous electrolytes are not useful tool for the accurate estimation of PMI.

After Vitreous humor Cerebrospinal fluid (CSF) is the most frequent body fluid, which is studied for the estimation of PMI. Following are the important studies which are made to determine PMI using CSF.

Mason, Klyne & Lennox [33] had studied the potassium levels in (CSF) after death. They collected cerebrospinal fluid samples from 46 different dead bodies. Flame photometer was used for potassium detection. They made a regression formula and from that they concluded that the CSF potassium level immediately rise after death. The concentration increases during the period of one and a half to 70 h after death.

Finehout *et al*. [34] did a study on proteomic analysis of CSF changes related to post-mortem interval. They collected both CSF samples of ante-mortem and post-mortem. The post-mortem interval was 1.5 to 9.5 h. They used 2D Electrophoresis for the analysis. They demonstrated that the mean % volume of creatine kinase B increases 12 – fold in post-mortem CSF compared with ante-mortem CSF from the same individual. They also observed increase in several of the proteins present in postmortem CSF.

Yadav *et al*. [35] worked on CSF electrolyte concentration in Bhopal region of central India for the determination of time since death. They studied 100 medico-legal cases with known time of death. They studied the age group of 15-70 years. CSF samples were analyzed by Flame photometer. They observed that concentration of potassium increased with the increase in post-mortem interval, while sodium level decreased with increasing post-mortem interval. Concentration of potassium showed a positive statistically significant correlation of 0.934. On the other hand, sodium values showed a significant negative correlation of -0.868. They concluded that collection of CSF from the lateral ventricles gives clear sample and concentration of sodium, potassium and their ration from CSF provide a significant parameter to estimate the time since death. They also concluded that external factors like, temperature affects the estimation. So, such factors should be taken in consideration while calculating PMI. They stated that the ratio of sodium and potassium concentration is a better parameter for predicting time since death compared to PMI estimation from sodium or potassium values alone.

Jekins [19] studied thirty-eight autopsy cases for the determination of urea level in cerebrospinal fluid after death. He distributed each sample in four different groups. He concluded that post-mortem CSF urea levels at varying periods after death were found to correlate closely with ante-mortem blood levels.

D Wyler, Marty & W Bar [36] has studied the cell content of cerebrospinal fluid after death. They collected 35 samples of CSF. They had kept the samples at two different temperatures of 4˚c and 20˚c. They noted that there is no significant rise in cell content with increasing post-mortem interval.

A.P. Dongre and R.V. Bardale [37] have studied the changes in potassium level of CSF. The CSF samples were drained from 100 cases at a fixed interval of six hour i.e., at 6, 12, & 18 hours after death. The age group ranged from 11 year to 80 year. There were 73 men and 27 women. The time of death was known and was confirmed from doctors, relatives, police personal and death summary. The potassium was analysed on flame photometer. They observed that there was an increase of potassium concentration with the increase of time interval. They also studied the concentration of inorganic phosphorus using semiautomatic analyzer. The mean increase of inorganic phosphorus concentration in the postmortem interval is relatively linear with the time and the rise is statistically significant ($p < 0.001$). The 95 % limits of confidence of the CSF inorganic phosphorus at 6-hour postmortem interval are ± 5.97 mg/dL, by 12^{th} hour it laid ± 7.72 mg/dL, and at 18^{th} hour postmortem interval are ± 10.01 mg/dL. Dongre [34] also studied the sodium concentration in post-mortem CSF. Sodium was analyzed using flame photometry. There is a decrease of sodium concentration with increasing post-mortem interval. Dongre [34] observed that there is a decrease in the concentration of chloride after death. In their study glucose analysis was also done using semi-automatic analyzer. They noticed that the concentration of glucose decreases after death.

A K Parmar and S K Menon worked on CSF albumin. They stated that albumin present in CSF undergoes changes. They found a linear decrease in albumin concentration from 2 h to 72 h after death. Their study indicated an important relationship between albumin concentration and post-mortem interval with an error of ±1–5 h [38].

Now a day synovial fluid and pericardial fluid are trending in use for the estimation of PMI. Somehow not much work has been done on both fluids. The following researches highlight the importance of the different body fluids as well as different organs for the estimation of PMI.

N Sheikh [39] worked on cadaveric synovial fluid for estimation of post-mortem interval. He studied 123 autopsy cases for the determination of PMI. Potassium concentration of the synovial fluid is measured by flame photometry. All cases were distributed in age and sex group. From the statistical analysis he concluded that potassium concentration rise up in a linear way with increasing time interval up to 48 hrs. N Shaikh [40] also studied the level of sodium and glucose in cadaveric synovial fluid. He stated that both sodium and glucose levels have irregular change with increasing PMI. He found no significant correlation between the levels of sodium and glucose and PMI.

Nilesh J, Rajesh B, and Anand D [41] did a study on determination of post-mortem interval by analysis of synovial fluid and vitreous humor. In this research 154 cases were examined. Concentration of sodium, potassium and chloride was analyzed by Ion selective method, while calcium, creatinine, glucose and urea were analyzed by semiautomatic analyzer. They stated that sodium, calcium,

creatinine, urea present in both fluids did not show any significant change after death. While chloride shows decrease after death but its mean value is not so significant. Potassium and glucose concentration in both fluids significantly changed with increasing PMI. They concluded that potassium present in both fluids shows an increase with increase in interval and this change in synovial fluid is more significant than in vitreous humor.

Dalbir S *et al.* [42] studied the electrolyte concentration in pericardial fluid for the estimation of time since death. The study was done on 311 dead bodies. Statistical analysis was carried out by using double logarithmic model. They stated that there is no significant relationship between the pericardial electrolytes and PMI. But when the data was transformed to a double logarithmic scale; potassium, sodium / potassium ratio and phosphorus shows noteworthy relationship with PMI. It is also concluded that accurate estimation of PMI can be made by measuring the concentration of potassium, chloride and phosphorus present in pericardial fluid. They concluded that pericardial fluid has an advantage over vitreous humor and CSF as it is easily available in large volume.

Shiwei, Gaowen, Ronald, Zhen-yuan [47] did a study on the changes in ATP and its degradation products in relation to estimate time since death. Mainly brain, spleen and kidney of rats were used for the analysis. They divided the samples in to two groups, in which one group's samples were stored at 4˚C and other group's samples were stored at 20˚C. HPLC was used for the analysis. The statistical analysis was done from the result. They stated that the K value (% all the ATP breakdown products) from each sample shows a linear increase with an increase in PMI. K value of the samples is a useful index for the estimation of PMI but still this study is limited up to the middle stage after death as in decomposition period the K value of the samples is not remain stable.

Conclusion

In this modern era, with the faster growing world, the techniques used for determination of post-mortem interval needs to be rapid, sensitive and more precise. The traditional methods like cooling of the body, rigor mortis, post-mortem lividity, decomposition, etc... have been used for the same purpose for the past several years. And they have played a very important role in the growth of accuracy for determination of post-mortem interval. But still such traditional methods are a type of superficial as they failed to give an accurate time of death. As the biochemical methods have entered the world of forensic medicine, it has altered the face of estimation of post-mortem interval. Biochemical methods are rapid, sensitive, stable and accurate. Traditional methods are affected by the external factors and hence failed to give precise idea about the time of death. But this nuisance has been defeated by the use of modern biochemical techniques.

Biochemical methods can be improved by making it cost effective and by incorporating with latest technologies like highly precise instruments and use of nanotechnology. Also the statistical analysis of the data should be employed in the routine practice for more precise result. If such improvement is achieved then it will not only an achievement in the world of forensic science but it will also be more beneficial to the court of law.

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