

# Diagnostic Analysis of Electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>+2</sup> and PO<sup>-3</sup><sub>4</sub>) in Cadaveric Synovial Fluid from Knee Joint to Estimate Postmortem Interval

AATIQA ABBAS<sup>1</sup>, AROOJ FAROOQ<sup>2</sup>, MUHAMMAD ALI FAROOQ<sup>3</sup><sup>1,2</sup>MBBS, M.Phil Forensic Medicine & Toxicology, KEMU, Lahore<sup>3</sup>MBBS (MBBS, MC Mirpur) House officer at DHQ Mirpur, AJKCorrespondence to: Arooj Farooq, Email: [draroojsifat@gmail.com](mailto:draroojsifat@gmail.com), Cell: +92 336 4194109

## ABSTRACT:

Accurate determination of Time since death (TSD) has always been a challenge to forensic pathologists. Due to non-precise results using physical methods to estimate time of death, focus has been shifted to biochemical methods using body fluids. Previously, body fluids like cerebrospinal fluid (CSF) and vitreous humour have been used to determine postmortem interval (PMI), but in cases of head and ocular injuries respectively, they can be rendered useless. Synovial fluid is a well-preserved, compartmentalized fluid and hence, can be used to estimate PMI. The aim of this study was to determine death interval based on synovial fluid electrolytes levels (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>+2</sup>, PO<sup>-3</sup><sub>4</sub>) and to assess its reliability for future studies.

**Study design:** It was a correlational study.

**Study settings:** The research was conducted at department of Forensic Medicine King Edward Medical University, Mayo Hospital Lahore, from August 2018 to August 2019.

**Methodology:** PMI was regressed on synovial fluid electrolytes and regression formulae were formulated. Significant positive correlation was found between time since death and K<sup>+</sup> ion concentration using regression formula.

**Results:** However, there was negative relationship between PMI and Na<sup>+</sup> and no significant relationship was found between TSD and Cl<sup>-</sup>, Mg<sup>+2</sup> and PO<sup>-3</sup><sub>4</sub> concentrations. The regression model of K<sup>+</sup> gave a 97.9% accuracy and a correlation coefficient of 0.978 which showed the model using K<sup>+</sup> values in synovial fluid is the most accurate one.

**Conclusion:** The concentration of potassium ions increases with increasing time of death whereas, that of sodium decreases with increasing PMI. However, magnesium, chloride and phosphate concentrations remain unchanged with time interval.

**Keywords:** sodium, magnesium, chloride, phosphate, electrolytes, synovial fluid

## INTRODUCTION

Estimating PMI is of paramount importance in medicolegal investigations. TSD or PMI is 'the time period between death and examination of the body'.<sup>1</sup> Evidence for determining TSD is obtained from i.e. corporeal, environmental conditions. All these parameters give an estimation of time interval between death and injury.<sup>2</sup> The goal for determining TSD at the crime scene is to have an idea regarding time of assault and for narrowing down the number of assailants.<sup>3</sup> Physical changes in the body (Algor mortis, Rigor mortis, livor mortis etc.) produce erroneous results as they get affected by external environmental conditions.<sup>4</sup> New approach for determination of time since death includes chemical alterations in certain ions in body fluid after death as they cannot be altered.<sup>5</sup> Chemical changes can be seen in blood, CSF, aqueous humour, vitreous humour, and synovial fluid right away or shortly after death. Until the body decomposes, these changes proceed in a systematic manner.<sup>6</sup>

Uptil now, synovial fluid has been investigated for determining alcohol concentration and drug distribution, but more recent studies have revealed that it can be used to estimate PMI compared to other fluids like CSF or vitreous humour in cases where there is head or ocular trauma respectively.<sup>7</sup> Synovial fluid is preferred over other body fluids as it is a well preserved fluid even after death and is less subjected to contamination and putrefaction because of its anatomical compartmentalization in human body.<sup>8</sup> Also, chemical changes take place very slowly in it as compared to blood and CSF. The purpose of this study was to evaluate the reliability of synovial fluid Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>+2</sup> and PO<sup>-3</sup><sub>4</sub> concentrations for determining PMI and to assess the accuracy for future use of synovial fluid as a precise PMI estimation medium.

## MATERIAL AND METHODS

In this study, the subjects were the cadavers which were brought in the department of Forensic Medicine & Toxicology, KEMU, LHR for

postmortem examination. Total number of samples was 153, out of which 113 cases were males and 40 were females. Cases were divided into total five groups of twenty hours each based on death interval. Only cases with known death intervals (circumstantial evidence and police documents) were taken. Others with traumatic knee injuries, metabolic disorders, burnt bodies, discoloured or hemorrhagic samples were excluded. The temperature was kept twenty to thirty degrees before samples were collected. Two milliliters of synovial fluid was aspirated using 18 gauge 10 ml needle and supra patellar pouch was punctured laterally just below the patella in supine position. Analysis was done immediately after synovial fluid was aspirated. Prior to analysis, the sample fluids were centrifuged at 3500 revolution per minute (rpm) for 10 minutes and then the supernatant was used for analysis.<sup>9</sup> Parameters (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>+2</sup>, PO<sup>-3</sup><sub>4</sub>) were measured in synovial fluid using an auto-analyzer by ion –selective method. SPSS-23 was used to determine the relationship between synovium concentrations of all indicators and PMI. Linear regression formula was used for statistical analysis by SPSS 23.

## RESULTS AND OBSERVATIONS

Postmortem interval ranged from 0-96 hours. The values of all five parameters (electrolytes) analyzed in synovial fluid are stated in table no.1. TSD and K<sup>+</sup> have a strong positive correlation (r=0.978) fig.1 was found. However, there was a negative correlation between PMI and Na<sup>+</sup> (r=0.976) fig.2, When comparing TSD to Cl<sup>-</sup> (r=0.012), Mg<sup>+2</sup> (r=0.063), or PO<sup>-3</sup><sub>4</sub> (r=0.145), no significant associations were seen. Using synovial fluid K<sup>+</sup> and Na<sup>+</sup> values, these are the regression equations used to estimate PMI:

$$\text{PMI} = -60.23 + 8.828 (\text{k in mmol/l}).$$

$$\text{PMI} = 119.9 - 0.9389 (\text{Na in mmol/l}).$$

Table 1: parameters values in synovial fluid

	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Mg <sup>+2</sup>	PO <sup>-3</sup> <sub>4</sub>
Minimum	38mmol/l	5.8mmol/l	98mmol/l	0.75mmol/l	1.1mmol/l
Maximum	134mmol/l	17.4mmol/l	101mmol/l	0.8mmol/l	1.2mmol/l
Mean	80.4mmol/l	11.86mmol/l	100.2mmol/l	0.82mmol/l	1.03mmol/l
Std.deviation	30.7mmol/l	3.20mmol/l	2.19mmol/l	0.07mmol/l	0.13mmol/l

The regression model using  $K^+$  ions concentration in synovial fluid proved to be more accurate than  $Na^+$  ions concentration as its correlation coefficient ( $R^2$ ) value was 97.9% while for  $Na^+$  its 97.6%. Whereas,  $R^2$  values of  $Cl^-$ ,  $Mg^{+2}$  and  $PO_4^{-3}$  were 1.2%, 6.3% and 14.5% respectively.

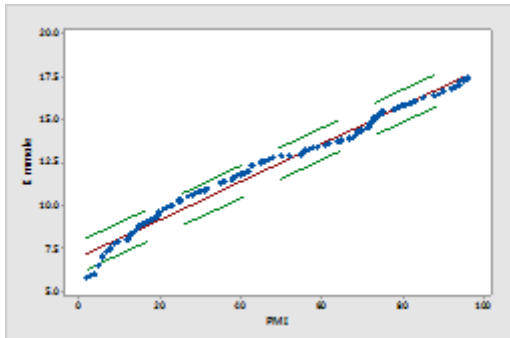


Fig 1: Scatter Plot between PMI and  $K^+$

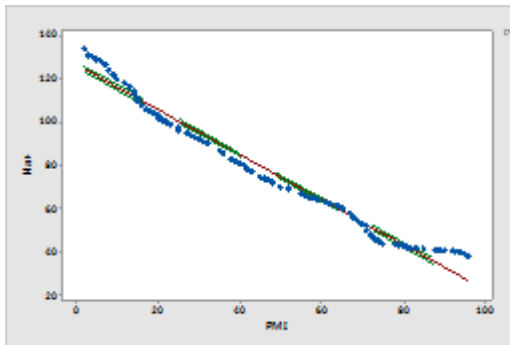


Fig 2: Scatterplot between PMI and  $Na^+$  mmol/l

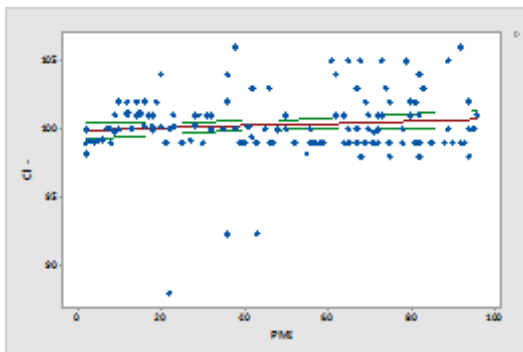


Fig 3: Scatterplot between PMI and  $Cl^-$  mmol/l

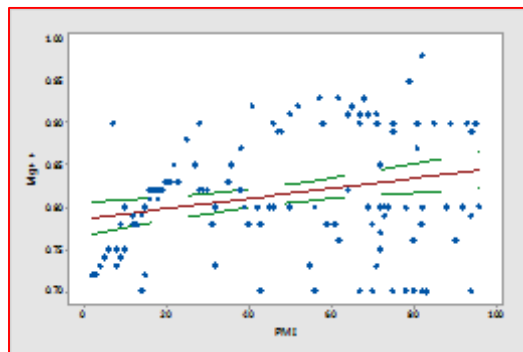
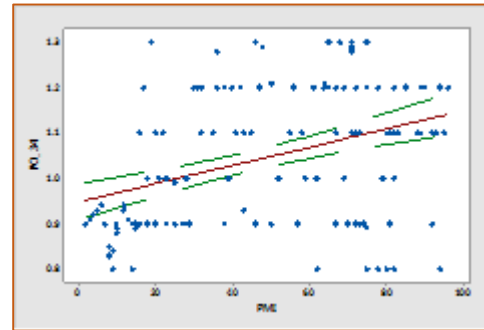


Fig 4: Scatterplot between PMI and  $Mg^{+2}$  mmol/l



Scatterplot between PMI and  $PO_4^{-3}$

## DISCUSSION

There was a wide range of  $K^+$  concentrations in synovial fluid, with a mean of 11.86 mmol/l and a standard deviation of 3.20 mmol/l. TSD and  $K^+$  content in synovium were found to have a positive correlation. Researchers in the fields of Madea<sup>10</sup>, Sahoo<sup>11</sup> and Sheikh<sup>12</sup>, Tumram<sup>13</sup> and SiddhamTumram<sup>13</sup> all found the same thing. This study found that the mean and standard deviation of the  $Na^+$  concentration in synovial fluid were 8.4 and 30.7 millimoles per litre, respectively. Sodium had a negative linear connection with PMI, and these findings are in line with those of previous research by Madea<sup>10</sup>, Sahoo<sup>11</sup>, Sheikh<sup>12</sup>, Tumram<sup>13</sup>, and Siddhamsetty<sup>14</sup> as well. From 98mmol/l to 101mmol/l, synovial fluid  $Cl^-$  concentrations ranged from 100.28mmol/l to 2.19%, with the mean being 100.28 and the standard deviation being 2.19mmol/l. Scatterplot showed that there was no relation between its concentration and TSD. Madea<sup>10</sup> and Tumram<sup>13</sup> deduced same results when they first studied this parameter in synovial fluid.  $Mg^{+2}$  and  $PO_4^{-3}$  levels in synovial fluid ranged from 0.75mmol/l to 0.8mmol/l and 1.1mmol/l to 1.2mmol/l respectively with mean and std. deviation of 0.82mmol/l and 0.07mmol/l for magnesium and a mean and std. deviation of 1.03mmol/l and 0.132mmol/l for phosphate with their scatterplot showing that there was no significant correlation between  $Mg^{+2}$ ,  $PO_4^{-3}$  concentrations in synovial fluid and PMI. In the past, most of the notable researches by scholars were conducted using  $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $Ca^{+2}$  and glucose concentrations in synovial fluid but, none of them analyzed  $Mg^{+2}$  and  $PO_4^{-3}$  ions. So, in existent study, both these electrolytes have been included for analysis in order to procure a correlation between their concentration and TSD.

After death, when there is complete cessation of respiratory and circulatory systems, the metabolic activities continue at cellular level for variable amount of time.<sup>14</sup> The postmortem changes in biochemical electrolytes especially  $K^+$  occurs mainly due to anaerobic glycolysis resulting in loss of active membrane transport as well as selective membrane permeability, resulting in passive diffusion of electrolytes in different body compartments according to their concentration gradients.<sup>15</sup> Autolysis proceeds faster in blood than in CSF and even slower in synovial fluid and vitreous humour as they are well-preserved.<sup>16</sup> Hence, both these fluids are preferred for late postmortem chemical changes to determine PMI.

## CONCLUSION

The present study had been carried out to correlate changes in concentration of electrolytes in synovial fluid from knee joint and time since death with conclusion that the concentration of potassium ions increases with increasing time of death whereas, that of sodium decreases with increasing PMI. However, magnesium, chloride and phosphate concentrations remain unchanged with time interval. Irrespective of technical methodology advancements for estimation of PMI, the accuracy of these still leaves room for improvement. So there is need to develop more precise techniques as this will prove beneficial in field of forensics and will also eliminate chances of error as well.

**Recommendations:** As medicolegal cases require a serious need for an approximate time since death estimation, so, this aforementioned study can help in this regard and can be recommended to Punjab Forensic Science Agency to incorporate the biochemical analysis of synovial fluid electrolytes concentration for a precise estimation of PMI which can further help out the investigating agencies.

## REFERENCES

1. Postmortem Interval [Updated October 2018 Cited December 2018] Available from: [https://en.m.wikipedia.org/wiki/post-mortem\\_interval](https://en.m.wikipedia.org/wiki/post-mortem_interval).
2. Sankova M, Racanska M. Molecular Genetics and determination of time since death-short communication.2016; 61(3):28-9.
3. Mathur A, Agarwal YK. An overview of methods used of estimation of time since death. Aust J Forensic Sci.2011; 43(4):275-85.
4. Henssge C, Madea B. Estimation of time since death. Forensic Sci Int.2007;165(2-3):182-84.
5. Coe JI. Postmortem chemistries on blood with particular reference to urea nitrogen, electrolytes and bilirubin. J Forensic Sci.1974; 19(1):33-42.
6. Henry JB, Smith FA. Estimation of the Postmortem Interval by chemical means. Am J Forensic Med Pathol.1980; 1(4):341-47.
7. Al-Alousi LM. A study of the postmortem cooling curve in 117 forensic cases. Forensic Sci Int.2002; 125(2-3):237-244.
8. Baccino E, Martin L, Schuliar Y, Guillatian P, Le Rhun M et al. Outer ear temperature and time of death. Forensic Sci Int. 1996; 83(2):133-146.
9. Tumram NK, Bardale RV, Dongre AP. Postmortem analysis of synovial fluid and vitreous humour for determination of death interval: a comparative study. Forensic Sci Int.2011; 204(1):186-90.
10. Madea B, Kreuser C, Banaschak S. Postmortem biochemical examination of synovial fluid – a preliminary study. Forensic Sci int 2001;118(1):29–35.
11. Sahoo PC, Mohanty NK. Study of sodium, potassium and glucose level in synovial fluid for estimation of time since death. J Forensic med Toxicol 1998;15(14):14-6.
12. Sheikh NA. estimation of postmortem interval according to time course of potassium ion activity in cadaveric synovial fluid. Indian J Forensic Med Toxicol 2007;1(2):45-9.
13. Tumram NK, Ambade VN, Dongre AP. Thanatochemistry: Study of synovial fluid potassium. Alexandria Med J 2014 ;50(4):369-72.
14. Siddhamsetty AK, Verma SK, Kohli A, Verma A, Puri D, Singh A. Exploring time of death from potassium, sodium, chloride, glucose and calcium analysis of postmortem synovial fluid in semi-arid climate. J Forensic Leg Med.2014;28(1):11-14.
15. Coe JI. Postmortem chemistries on blood with particular reference to urea nitrogen, electrolytes and bilirubin. J Forensic Sci.1974; 19(1):33-42.
16. Madea B, Henssge C. Forensic Medicine: clinical and pathological aspects. 10. Time since death.2<sup>nd</sup> ed. London; 2003.p.91-114.