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Original Research Paper

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ESTIMATION OF SERUM CALCIUM & PHOSPHATE LEVELS AND THEIR CORRELATION WITH THE TIME SINCE DEATH IN AUTOPSY CASES

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ABSTRACT Estimation of fairly accurate time since death from post-mortem changes still remains an important but difficult task to perform by almost every autopsy surgeon in Indian medico legal scenario. The aim of the present study was to evaluate changes in the levels of Calcium and Phosphate ions in blood serum of autopsy cases and find their role in determination of time since death. Blood sample was collected from the 200 dead bodies which were brought to Pt. B.D. Sharma Post Graduate Institute of Medical Sciences Rohtak, Haryana during the period of December 2017 to November 2018. The blood samples were collected and analysed using auto analyser to minimise the errors. The results showed statistically significant relation between serum calcium, serum phosphate and time since death up to 72 hours. Statistical correlation constant and P value for calcium and phosphate was ($r = -0.203^*$, $p = 0.004$) and ($r = 0.381^*$, $p = 0.001$) respectively.

KEYWORDS : Time since death, Electrolytes level, Calcium, Phosphate, Blood serum.

INTRODUCTION:

Estimation of time since death is one of the most important objective of post-mortem examination. Accurate estimation of time since death continues to be a major problem for the Forensic Pathologist and its determination plays an important and vital role in medico-legal cases because of the fact that Forensic Experts are very often required to answer questions relating to time since death in the courts of law. For investigation of crime and the implementation of justice it is very important to determine the interval between death and the time of post-mortem interval.

Accurate determination of post mortem interval, applied not only to civil law, in which ascertaining the exact time of death is of practical necessity in settling family, social and business matters but also to criminal law, where the accurate determination of time since death may either exclude and include a suspect or accused of particular homicide. [1]

In an attempt to find more reliable and precise parameters to estimate post mortem interval, many researchers have employed a variety of physical and chemical methods but most of these methods become useful supplementary means rather than replacing the traditional triad of Algor, livor and rigor mortis.

Autopsy surgeons continue to rely on age-old subjective methods of observing the degree and chronological staging of external as well as visceral post-mortem somatic changes like cooling of the body, loss of corneal reflex, changes in the eye, rigor mortis, hypostasis, signs of decomposition like mummification, adipocere formation, maggots infestation etc corroborated with circumstantial evidences for estimation of time since death. [2] Some clue about time of death is also gathered from the condition of food in stomach, intestine and urine in bladder. But the main problem with these age old trusted methods is there variability and inconsistency in finding the approximate time since death as almost all of these depends on the external surrounding. [3]

Post-mortem biochemistry is an important, but seldom practiced tool in death investigations. Biochemical analyses of different biological fluids such as blood, vitreous humour, urine, cerebrospinal, synovial, pleural and pericardial fluids are not only important in cases of sudden natural deaths, but also in estimation of time since death, in detection of various poisons, and for better understanding of pathophysiology of disease process. [4]

During the biochemical investigation of cadaveric body fluids, agonal and post-mortem interference should be taken into consideration. The use of biochemical parameters in post-mortem diagnosis requires knowledge of changes caused by autolysis, a factor closely linked to the post-mortem interval. Moreover, the distribution of these biochemical elements in different fluids of the cadaver depends on the origin and location of tissue damage. In this study we tend to find the role of electrolytes i.e. calcium and phosphate ions in blood serum in determination of time since death in medico-legal autopsy cases which brought to us for autopsy. As tests for electrolytes is comparatively cheap and readily available compare to various tests for other metabolites like lactic acid, hypoxanthine, uric acid, ammonia, NADH, formic acid etc. which are costly and mostly unavailable. With this study our main moto was to find some easily reproducible results which are cost effective and less dependent on external factors to answer the mystery of calculating time since death without error.

OBJECTIVES:

To measure the level of Calcium and Phosphate ions in the blood serum of autopsy cases and to find the changes in concentrations of serum calcium and Phosphate ions with passage of time after death.

MATERIAL AND METHOD:

The present study was conducted in the department of Forensic Medicine in collaboration with Department of Biochemistry at Pt. B.D. Sharma Post Graduate institute of medical sciences, Rohtak.

A total of 200 cases which were brought to the Department of Forensic Medicine of Pt. B.D. Sharma PGIMS, Rohtak for medicolegal autopsy from December 2017 to November 2018 were included in the study. Informed consent was taken from the next kin (close relatives) of the deceased and from the accompanying police official in case of autopsies conducted on unknown dead bodies for extraction of blood. The information of each case was recorded in a proforma including date, PMR number, age, sex, stature, time since death and cause of death. Blood samples were taken for recording physical characteristics and estimation of electrolyte (Calcium and Phosphate) concentration. The study was done after obtaining clearance from institutional ethical committee.

Inclusion Criteria: Cases with known time since death confirmed by treating doctors, relatives, police personnel and death summary from hospital documents.

Exclusion Criteria: Cases with known history of tumours or metastasis and cases where process of advance puterification already started i.e. greater than 72 hours.

Procedure: The blood sample was collected from medicolegal autopsy cases brought to the mortuary of Pt. B.D. Sharma PGIMS, Rohtak whose time since death was confirmed from doctors, relatives, police personnel and hospital death summary and cases were divided into eight groups according to cause / manner of death i.e. Road side accidents, Burns, Poisoning, Snake bite, Chronic illness, Asphyxia, Trauma other than RSA and Natural causes.

Ten (10) ml blood was collected in a plain vacutainer from femoral vein by aseptic means after exposing the femoral vein by dissection and clamping or ligating it proximal to the collection site. when the sample was not collected from femoral vein, blood was taken from internal jugular vein. In case of repeated failure sample was withdrawn directly from right ventricle of the heart.

The blood serum samples were analysed immediately. The samples were centrifuged at room temperature at 500 revolutions per minute for 5 minutes. Supernatant fluid was used for analysis. Electrolyte analyser instrument was standardized according to protocol provided by the manufacturer of the instrument and concentrations of electrolytes (calcium and phosphate) were measured and recorded on a Performa.

Data Analysis: Results were expressed as mean ± SD. The data were collected and entered in the MS EXCEL spread sheet, coded appropriately and then the data were subjected to statistical analysis using ANOVA test along with Pearson co-relation by application of SPSS 20 (Statistical package for the social sciences) software as per study objective.

OBSERVATIONS:

Table 1 shows male subjects were more in number than female in the present study. In males and females maximum number of the subjects belongs to age group 21 to 30 years and the subjects from age group 01 to 10 years were minimum. Table 2 depicts that the maximum number of subjects studied were within the 24 hour of death and highest were between 13 to 24 hours.

Table 3 shows that In maximum number of subjects cause of death was Road side accidents i.e. 113 (56.50%). and in their sub group injuries and their complications i.e. 61 (30.50%) were maximum followed by head injury 51 (25.50%) cases.

Table 4 depicts that out of 200 cases, Mean blood serum calcium value was found maximum in cases died due to natural causes followed by asphyxia cases and least in trauma other than RSA cases. The p value came out to be 0.061 ($p>0.05$) which showed that there was no statistical association between blood serum calcium and cause of death or we can say that change in blood serum calcium level does not depend on cause of death. After applying ANOVA test, F value = 1.969 and df = 7.

Mean blood serum phosphate value was found maximum in cases of trauma other than RSA followed by chronic illness cases and least in cases with death due to natural cause. The p value came out to be 0.196($p>0.05$) which showed that there was no statistical association between blood serum phosphate and cause of death or we can say that change in blood serum phosphate level does not depend on cause of death. After applying ANOVA test, F value = 1.427 and df = 7.

Table 5 depicts that the mean level of phosphate in the blood serum was regularly **increasing** with passage of time. After applying one-way ANOVA test, p value came out to be $p<0.01$ which showed that there was a strong correlation between the increase of phosphate level in blood serum and time since death (F value = 8.865 and df = 4) and that is highly statistical significant.

It has been observed that the mean level of calcium in the blood serum was **increasing till 24 hours then becomes variable** with passage of time. After applying one-way ANOVA test, p value came out to be 0.031 ($p<0.05$) which showed that there was statistical significant correlation between calcium level in blood serum and time since death (F value = 2.716 and df = 4).

Table 6 showed correlation of blood serum electrolytes with time since death. Time since death has demonstrated positive correlation with Phosphate in blood serum ($r=0.381$, $p=0.001$) which was found statistically significant.

It has been also observed that time since death has negative correlation with calcium in blood serum ($r=-0.203$, $p=0.004$) which was found statistically significant.

Table 1: Gender wise distribution of cases as per age group.

Age Group (Years)	Male (%)	Female (%)	Total (%)
01-10	01 (0.60)	01 (02.40)	02 (01.00)
11-20	16 (10.10)	06 (14.60)	22 (11.00)
21-30	41 (25.80)	11 (26.80)	52 (26.00)
31-40	29 (18.20)	07 (17.10)	36 (18.00)
41-50	33 (20.80)	04 (09.80)	37 (18.50)
51-60	21 (13.20)	05 (12.20)	26 (13.00)
61-70	15 (09.40)	04 (09.80)	19 (09.50)
>71	03 (01.90)	03 (07.30)	06 (03.00)
Total	159 (100.00)	41 (100.00)	200 (100.00)

Table 2: Gender wise distribution of cases as per time since death.

Time since death (hours)	Male (%)	Female (%)	Total (%)
01-12	41 (25.79)	09 (21.94)	50 (25)
13-24	90 (56.60)	22 (53.66)	112 (56)
25-36	17 (10.69)	05 (12.20)	22 (11)
37-48	07 (04.40)	03 (07.32)	10 (05)
49-72	04 (02.52)	02 (04.88)	06 (03)
Total	159 (100)	41 (100)	200 (100)

Table 3: Distribution of Cases According to Cause of Death.

Cause of death	Sub Groups	Number of cases	Percent of cases (%)	Total
(I) Road side Accident	Head injury	51	25.50	113 (56.50)
	Injuries and their Complications	61	30.50	
	Haemorrhagic shock	01	0.50	

(II) Burn		07	03.50	07 (03.50)
(III) Poisoning	Aluminium Phosphide Poison	01	0.50	51 (25.50)
	Methyl Alcohol Poison	01	0.50	
	Organophosphate Poison	02	01.00	
	Poison (Misc.)	47	23.50	
(IV) Snake Bite		03	01.50	03 (1.5)
(V) Chronic illness	Acute renal failure	03	01.50	16 (8)
	Renal failure diabetic	05	02.50	
	Septic Shock	03	01.50	
	Tuberculosis	02	01.00	
	Miscellaneous	03	01.50	
(VI) Asphyxia	Aspiration	02	01	06 (3)
	Hanging	04	02	
(VII) Trauma other than RSA	Uterine rupture	01	0.50	1 (0.50)
(VIII) Natural Cause		03	01.50	3 (01.50)
Total		200	100	200 (100)

Table 4: Mean levels of calcium and phosphate electrolytes in blood serum of autopsy cases and their relation with different causes of death.

Cause of Death	No. of subjects N = 200	Blood Serum Calcium (mEq/l)	Blood Serum Phosphate (mEq/l)
RSA	113	9.79 ± 2.331	34.55 ± 12.768
Burn	7	9.69 ± 1.939	32.16 ± 8.107
Poison	51	10.72 ± 3.018	30.51 ± 10.625
Snake bite	3	10.43 ± 2.371	31.73 ± 12.133
Chronic illness	16	9.14 ± 2.122	35.94 ± 9.238
Asphyxia	6	10.88 ± 2.611	33.97 ± 7.983
Trauma other than RSA	1	6.30	58
Natural cause	3	12.83 ± 1.858	28.60 ± 8.884

Table 5: Mean levels of calcium and phosphate electrolytes in blood serum of autopsy cases and their relation with time since death up-to 72 hours.

Time since Death (hours)	No. of Subjects N = 200 (%)	Blood Serum Calcium (mEq/l)	Blood Serum Phosphate (mEq/l)
01-12	50 (25)	10.49 ± 2.067	27.59 ± 9.818
13-24	112 (56)	10.25 ± 2.782	33.45 ± 10.328
25-36	22 (11)	8.83 ± 1.876	40.06 ± 16.065
37-48	10 (5)	9.06 ± 2.398	41.47 ± 11.282
49-72	6 (3)	8.65 ± 2.484	44.85 ± 8.063

Table 6: Pearson Co-relation of Blood serum electrolytes with time since death.

Electrolytes	Pearson Correlation (r)	p-value
Blood serum Calcium	-0.203*	0.004
Blood serum Phosphate	0.381*	0.001

(*Correlation is significant at the 0.01 level.)

DISCUSSION :

Present study was conducted on 200 medicolegal autopsy cases which were bought to the Department of Forensic medicine at Pt. B.D. Sharma PGIMS, Rohtak Haryana. Amongst these 200 medicolegal autopsy cases male cases were more in number i.e 159 and female cases were 41. Autopsy cases from various age groups were included in the study to maintain uniformity and in order to remove age bias. Cases from age group 21 to 30 year were maximum in the study i.e. 52(26%) out of them 41(20.50%) cases were male and 11 (05.50%) cases were female.

Gender wise distribution of cases into group of time since death was done and it was observed that maximum cases were from the group of time since death from 13 to 24 hours i.e. 112 (56%) followed by the group of time since death from 01 to 12 hours i.e. 50 (25%).

The study cases were also categorised according to the cause of death i.e. Road side accident, burn, poisoning, snake bite, chronic illness, asphyxia, trauma other than RSA and natural causes. Amongst these eight groups, number of cases of road side accident category were maximum i.e. 113(56.50%) followed by poisoning cases 51(25.50%). These eight groups were further sub divided into sub groups amongst these, one of the sub group of road side accident i.e. injuries and their complications has maximum number of cases i.e. 61(30.50%) followed by sub group head injury i.e. 51(25.50%).

Relationship between cause of death and mean levels of blood serum electrolytes i.e. calcium and phosphate in the study group was also evaluated and mean levels of calcium and phosphate ions were compared with eight different causes of death group to find any significant statistical association in rise or fall of phosphate and calcium ion levels and cause of death by applying ANOVA test. No statistical association was found between changes in mean levels of blood serum electrolytes and cause of death.

Relationships between time since death up to 72 hours and mean levels of serum electrolytes i.e. calcium and phosphate was also analysed. It was observed that the mean level of phosphate in blood serum was regularly increasing with passage of time and it showed high statistical significance with time since death. It was also observed that though the level of blood serum calcium was increasing till 24 hours and after that it becomes variable but still statistical significant ($p<0.05$) correlation exists in blood serum calcium levels with time since death up to 72 hours.

This can be understood by the study of **Naussi and Davies**[5]on changes in phosphate compounds during the development and maintenance of rigor mortis where they showed that, as the muscle began to shorten the rate of Ca efflux increased and the ATP level fell from 3.80 to 0.0 moles per g. There was a lack of correspondence between ATP breakdown and appearance of inorganic phosphate, which can be explained by leakage of inorganic phosphate from the muscle and by an increase in the concentration of glucose 6-phosphate, fructose 6-phosphate, and glucose l-phosphate and study of **Howard et al** [6]on tumour lysis syndrome where they have explained that when cancer cells lyse, they release potassium, phosphorus, and nucleic acids, which are metabolized into hypoxanthine, then xanthine, and finally uric acid, an end product in humans. Likewise, **Eddington at el** [7] has done a study on serum phosphate and mortality in patients with chronic kidney disease where they observed significant rise in phosphate levels in chronically ill patients. Similarly **Zhu et al** [8]

Evaluated post-mortem serum calcium levels in relation to the causes of death and concluded that calcium levels in the heart and peripheral blood were significantly higher in salt water drowning compared with those of the other cause of death.

Present Study: The mean level of calcium in the blood serum was variable with passage of time. After applying one-way ANOVA test F value = 2.716 and p=0.031 which showed that there was no statistical significant correlation between the calcium level in blood serum and time since death. Time since death showed negative correlation with blood serum calcium r

= - 0.203** and p value 0.004 and mean level of phosphate in the blood serum was increasing with passage of time. After applying one-way ANOVA test F value = 8.865 and $p<0.01$ which showed that there was statistical significant correlation between the phosphate level in blood serum and time since death. Time since death showed positive correlation with blood serum phosphate r =0.381** and p value 0.001.

CONCLUSION

In the present study it was observed that mean value of blood serum calcium was variable with passage of time. Correlation of time since death with calcium in blood serum was negative and statistically significant. In the present study it was also observed that mean value of blood serum phosphate was increasing regularly with increase in time since death. Correlation of time since death with phosphate level in blood serum was found highly statistically significant.

From the above result we concluded that blood serum phosphate can be used as a reliable indicator for measuring time since death up to 72 hours. But the blood serum calcium can only be used as a reliable indicator for measuring time since death till 24 hours.

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