

Changes in Haemogram in Subjects After Death As A Tool To Estimate Time Passed Since Death

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Abstract: The present work was carried out in the Department of FMT, in collaboration with the Department of Haematology, NRSMCH to study the Changes in Haemogram in subjects after death as a tool to estimate Time Passed since Death. Total 84 cases were selected randomly at a known post-mortem interval ranging from 0 to 48 hours after death having age group of 2.5 years to 93 years. The cases were taken for the study are: a) The time of death is known b) Upto 48 hours after death and cases were not included in the study are: a) Decomposed dead bodies b) Exact time of death is not known c) Pre-existing haematological diseases. The blood sample collected in EDTA vial were analysed by Automated Cell Counter and evaluated the value of Haemoglobin, Haematocrit, total count of RBC, WBC, Platelet. The value of Haemoglobin and Haematocrit in postmortem blood were not correlated with time passed since death. Total number of RBC and WBC strongly correlated with Time passed since Death, though they are inversely correlated. Total number of Platelet strongly correlated with Time passed since Death.

Keywords: hemogram, time since death, haematocrit, RBC, WBC, Platelet

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I. Introduction

The times of death sometimes become extremely important. It is a question invariably asked by police officers to the Autopsy Surgeon, sometime with a touching faith in the accuracy of the estimate. But the fact is 'determining the time of death is extremely difficult & accuracy is impossible' as clearly mentioned by Bernard Knight in Legal aspect of Medical Practice, 4th Edition 1987, Churchill Livingstone Edinburgh. Estimation of time passed since death is one of the most important real time problems in medicolegal investigation. The forensic experts has to answer how much time passed since death after performing the autopsy to help the police in investigation . Accurate calculation of time elapsed since death is the potential to reveal many unfolded medicolegal mysteries. Though it is never possible to fix up the exact time of death by any of the postmortem findings of autopsy examination, yet some close and reasonable approximation of the time of death should always be aimed at.

The estimation of time since death is of utmost value both in civil as well as in criminal purpose. This data becomes particularly important in the criminal investigation when no eyewitness was present in the crime scene to exclude some suspects and to prevent taking alibi by some of the suspects.

The criminal cases where time passed since death is important are-

- i) To know when the crime took place as it gives the investigating Police Officer a starting point for their inquires and allow them to deal more efficiently with the information available.
- ii) It might enable to exclude some suspects and the search for the likely culprits.
- iii) To prove or disprove an alibi.
- iv) To check on the reliability of suspect's statement.

Similarly, to **the civil cases where also the postmortem interval is very important. These conditions are –**

- i) It might determine who will inherit the property.
- ii) Whether an insurance policy was in force.

The autopsy surgeon during Postmortem examination should keep record of all available data having bearing on this issue. Though it is never possible to fix up exact date and time of death by any findings of autopsy examination, yet some close and reasonable approximation of the time of death should always be tried for. It will rather be rash to pronounce the exact time of death in the witness box; and in an attempt to do so, there is every likelihood for the bounds of accuracy being overstepped and consequent injustice meted out. One

instead of giving a single estimates of the time since death, but should provide a range of times, between which the death was presumed to have taken place. The longer the postmortem interval, the wider is the range of estimate, i.e. the less accurate the estimate of the interval. In determining time of death, the Autopsy Surgeon should not over interpret what he sees and should not make dogmatic, unsupportable and potentially inaccurate statement. The range of time provided is at best an educated guess, based on knowledge and experience with little subjective error. It is very clear to see that medicolegal expert have to rely mostly and probably solely on age old subjective methods of observing the external as well as internal postmortem changes in the body that taken place after death like cooling of the body, rigor mortis, changes in the eye, hypostasis, maggot infestation, decomposition, or its alternative forms e.g. mummification, adipocere formation etc and to some extent on circumstantial evidences. The exact time of death cannot be fixed by any of the methods, only a range as to time of death can be opined because of various modifying factors like age, sex, occupation, cause of death, environmental conditions etc including individual variations.

Hence it has become extremely essential to re-explore other objective methods such as bio-chemical, histological, serological assays etc. An effort was made to ascertain whether it was practical and significant enough to estimate time since death by knowing quantitative serum enzyme changes. Obviously one has to keep in mind that since dealing with biological material like human corpse, blood sera etc. there have to be inherent biological variations in antemortem levels compared to the postmortem changes. We know that Blood in chambers of the heart and lumen of blood vessels get coagulated slowly after death within 4 to 6 hours; often it may not coagulate until putrefaction sets in. Within 2 to 3 minutes of blood shedding, fibrin filaments form and by 10 minutes approximately the shed blood gets clotted and by another 0 to 20 minutes, the serum may get expressed out.

Various studies have been done on the post mortem changes in the blood. Constituents such as blood pH, haematocrit values, electrolyte, enzymes etc to evaluate the correlation with time passed since death. It has been found that some constituent remain remarkably stable in the blood after death while others show varying degrees of changes. When change occurs, the alterations follow predictable patterns for certain substances while being totally erratic for others. A marked change occurs in postmortem blood as compared to antemortem values. Other body fluids such as cerebrospinal fluid, vitreous humor etc are also taken into consideration in various studies done by other research scholars. Thus it is expected that the data of Hemogram such as hematocrit value, number of blood cells (RBC, WBC, Platelet), hemoglobin level are correlated with time dependant cell death and may be taken as marker to estimate time passed since death. It is known that whatever parameters we use, they are subjected to a wide range of variations and are affected by various factors like age, sex, condition of the body, place, environmental condition, cause of death, diseases etc. So the parameters should be modified depending upon difference in place, seasons, individual characteristics so that time passed since death can be calculated in a narrow range in particular case. Due to these variations, the findings of various foreign workers who have worked in different atmospheric condition and places may not be helpful in solving the day-to-day problems faced by the forensic experts while doing postmortem examination at different places all over the world.

Again the climatic condition in different states of India and also in different parts of the same state differ so greatly that the findings in one state may not be useful for the other state or in different place of the same state. The change in morphology of blood cells are also subjected to wide variations and affected by various individual's internal and external factors viz. age, sex, condition of body, place, environmental condition, cause of death, diseases etc. Here in this study we have exercised utmost sincerity and care in dealing with every aspect of our topic for research. We deem it our joint success even if our work is able to make very small contribution to the ongoing research in the field.

Review of literature:

Estimation of the time of death is one of the most difficult job but inadequate facilities make the Forensic pathologist crippled. So Milton Helpem in his book "Autopsy- the Memories of Milton Helpem, the world's greatest medical detective" wrote 'Estimating the time of death is one of the most difficult and inaccurate techniques in forensic pathology'. Despite a wide range of variations affected by different factors observed in individual cases, postmortem changes are classified in such a manner so that postmortem interval can be estimated basing on time oriented following three headings-

- I. Immediate changes (stage of somatic death)
- II. Early changes (stage of molecular death)
- III. Late changes (stage of decomposition)

Changes after death vary with time since death and discussed below-

- I. Immediate change- These changes occur in the dead body at the time of somatic death and are as follows:
 - Insensibility and loss of voluntary power.

5-15	Neutrophils-cytoplasm change begins
7-12	Monocytes –cytoplasm change begins
8-10	Lysis of membrane of lymphocyte begins
8-15	Neutrophil-Nuclear change begins
10-12	Eosinophil-cytoplasm change begins
10-14	Eosinophil-Nuclear change begins
11-14	Lymphocyte-cytoplasm change begins
12-15	Monocyte- Nuclear change begins
14-17	Monocyte-complete lysis of cell wall
15	Lymphocyte –Nuclear change begins
18	Lysis of RBCs
18-20	Neutrophil-complete lysis of cell wall
21	No identifiable platelets
24-27	Identifiable degenerate lymphocytes only

Babapulle CJ^[7] et al of Dept of Forensic Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka in their study Cellular changes and time since death shows post-mortem cellular (chiefly nuclear) changes in the white blood cells of non-refrigerated cadavers could reliably be correlated with the time interval since death. Neutrophils degenerated rapidly; lymphocytes did so slowly; the Eosinophils and Monocytes degenerated at rates between these extremes. In cadaveric blood total counts of identifiable leucocytes on average dropped to zero by 84 hours, identifiable Eosinophils and Monocytes were first to 'disappear' (by 60 hours), followed by Neutrophils (by 66 hours), and finally lymphocytes: identifiable lymphocytes disappeared completely at or around 84 hours from the time of death.

Pentilla A and Laiho K^[8] worked on 123 cases of medico legal autopsies with post mortem time ranging from 1.7 hours to 270.4 hours. The corpses were kept at 4⁰C. The haematocrit value of the blood found to be increased after death. The haematocrit corrected red cell count and total white cell and platelet counts remained quite stable during whole post mortem range. Red cells were quite rapidly transformed from a discoid configuration to crumbled disc, echinocyte and spherocyte but no debris or burst cells configurations were seen. Rapid deterioration of the staining properties and marked morphological changes in many leucocytes occurred quite rapidly after death. Lymphocyte seemed to be the most resistant and the Basophils the least resistant to the effects of autolysis. Morphologically altered platelets and aggregates of them were seen in each cadaver. The present morphological observations and the quantitative results suggests that various cellular elements of the blood seem to be quite resistant to autolytic effects, and many cells apparently retain their viability for longer periods of time in the blood of cadavers kept at reduced temperature.

Dokgoz H^[9] et al of The Council of Forensic Medicine, Istanbul, Turkey done a study on Comparison of morphological changes in white blood cells after death and in vitro storage of blood for the estimation of postmortem interval .In this study, in vitro storage and postmortem changes of white blood cells were aimed to be compared within the given postmortem interval, and a follow-up study was carried out. Blood smears which were obtained from 10 non-refrigerated cadavers (experimental group) and from 40 hospital patients (control group) have been evaluated that Eosinophils and Monocytes were identifiable up to 72 hours, Neutrophils up to 96 hours and lymphocytes up to beyond 120 hours after death.

Aims and objectives:

To estimate the change of hemogram pattern after death with variation of time interval.

III. Materials And Methods

Study Area: The present study was conducted in the Department of Forensic Medicine in collaboration with Department of Hematology, NRS Medical College and Hospital. Blood sample collection was done in the Morgue of NRSMC&H. Complete Haemogram from blood sample was done in the Department of Haematology.

Study period: The study period extended from 1st August'2016 to 31st August'2017

Study duration: 12 months.

Study population: For the study blood sample was collected from the dead bodies coming to NRSMCH Morgue. The brief history of the case like the date and time of death, cause of death etc were collected from the inquest, the family or the relatives of the deceased. The dead bodies were selected depending upon certain inclusion criteria and exclusion criteria:

Inclusion criteria-

- a) The time of death is known.
- b) Up to 48 hours after death.

Exclusion criteria-

- a) Decomposed dead bodies.
- b) Exact time of death is not known because of any reason like brought dead cases.
- c) Pre-existing haematological diseases.

Sample size- This research work was carried out on 84 dead bodies belonging to both sexes, irrespective of age, in the morgue of NRSMCH. The blood samples were collected from those dead bodies in which cases known postmortem interval were 4 hours to 48 hours.

Sample design- The study is based on non-random purposive sampling.

Study design- It is a type of cross-sectional study.

Collection of sample- Postmortem blood sample were collected by piercing Femoral vessels, Common Carotid artery and on dissection from chambers of Heart with the help of 5ml disposable syringe. Blood is kept in EDTA vial for study.

Study technique- This study was done to estimate hematological parameters such as total count of RBC, WBC, Platelet, percentage of Hemoglobin and Hematocrit value.

Hematological Parameters: For hematological parameters the vial containing the EDTA preserved blood was taken to Hematology department immediately after collection from morgue. First of all it was observed whether there was any clotted blood in the EDTA vial, if so then the sample was discarded. Then in Automated Cell Counter the EDTA vial containing blood sample was brought before a metallic hollow probe. The probe sucks 0.2ml blood when command was given in the machine. Postmortem number was set as identification no for each blood sample. Automated Cell Counter generate a paper strip which contain the identification no, total count of RBC, WBC, Platelet, percentage of Haemoglobin, value of Haematocrit along with also show MCV, MCH, MCHC, R/W etc. After studying each sample the probe was washed by automated mechanism. Then only the next sample was put for analysis.

IV. Statistical Analysis

Collected data were analyzed and statistical test were done with the help of appropriate statistical tools.

Test for statistical significant was applied by using 't test' for analyzing the difference between the two Means, where $p < 0.05$ was considered significant.

The **t value** is after an estimation of a coefficient, the t-statistic for that coefficient is the ratio of the coefficient to its standard error. That can be tested against a t distribution to determine how probable it is that the true value of the coefficient is really zero. In statistical significance testing, the **p-value** is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true. One often "rejects the null hypothesis" when the p-value is less than the significance level α (Greek alpha), which is often 0.05 or 0.01. When the null hypothesis is rejected, the result is said to be statistically significant. In statistics, the number of **degrees of freedom** is the number of values in the final calculation of a statistic that are free to vary.

Correlation is used to establish and quantify the strength and direction of the relationship between two variables. Pearson correlation is used for interval or ratio scale data. Positive correlation between two variables means high values of one variable are associated with high values of other variables, where as negative correlation means high values of one variable are associated with low values of the other.

Consent: Consent is obtained from the next to kin of the deceased or from the legal guardians of the deceased.

Conflict of interest: there is no conflict of interest. The study has been conducted without revealing identification of deceased and there are no economical transactions or any monetary contribution from any person or agencies while conducting the study.

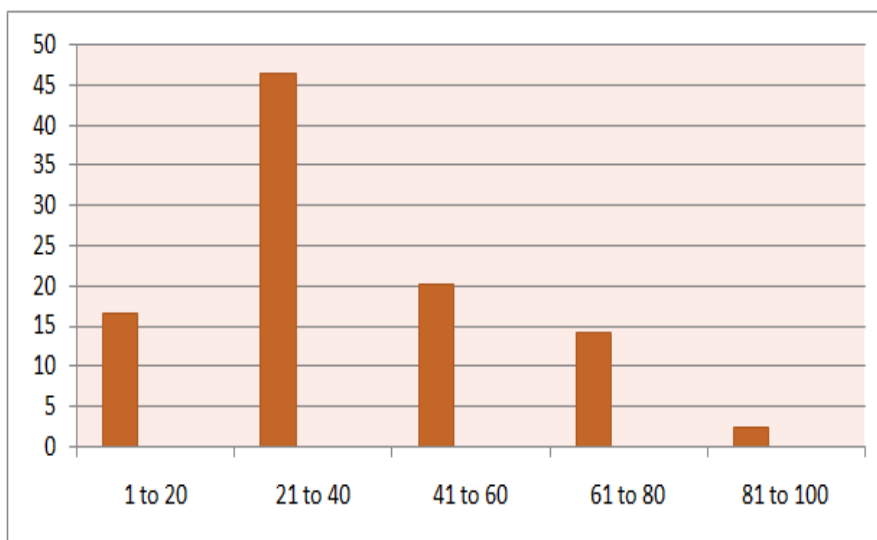
V. Results And Analysis

The blood samples for the present study were collected at random from 84 corpses brought for medicolegal autopsy to the morgue of Forensic and State Medicine Department, NRS Medical College and Hospital.

In the present study a total of 84 samples were collected from the corpses, among this 49 males and 35 females in the age group of 2.5 to 93 years. Out of these 84 cases 14(16.67%) were of 1 to 20 years, followed by 21 to 40 years which was 39(46.43%), 41 to 60 years, which was 17(20.24%), 61 to 80 years, which was 12(14.28%) and 81 to 100 years which was 2(2.38%) as shown in Table-I.

Table – I Age wise distribution of cases

AGE(Years)	NO OF CASES	% of cases
1 – 20	14	16.67
21 – 40	39	46.43
41 – 60	17	20.24
61 – 80	12	14.28
81 – 100	2	2.38
Total	84	100.00

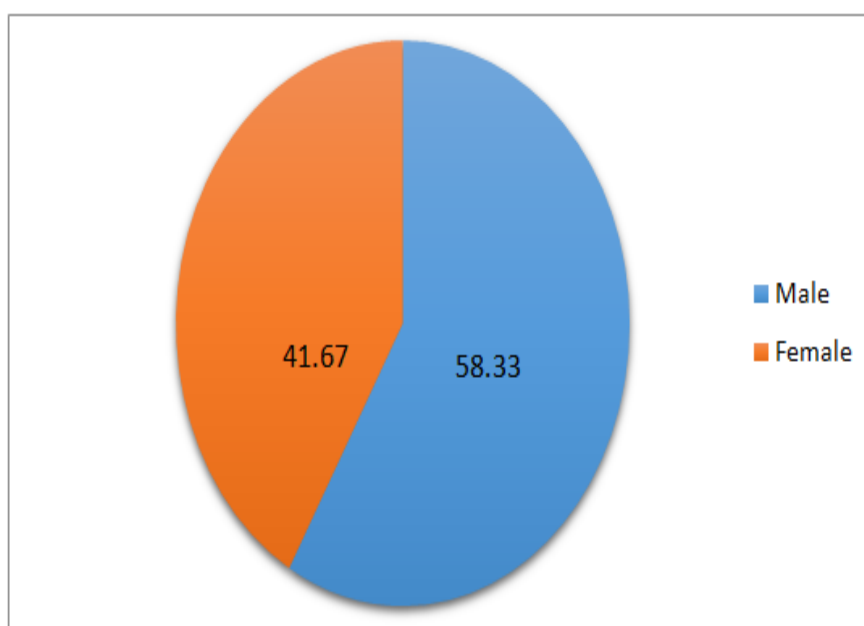


Diagrammatic Representation of cases based on Age.

- Sex wise distribution of the cases in the present study showed that among total 84 cases number of male subjects were 49 i.e.58.33% and number of female subjects were 35 i.e. 41.67%. It is obvious that in the present study males are predominant than the females.

Table – II Sex wise distribution of cases:

Sex	No of cases	% of cases
Male	49	58.33
Female	35	41.67
Total	84	100.00

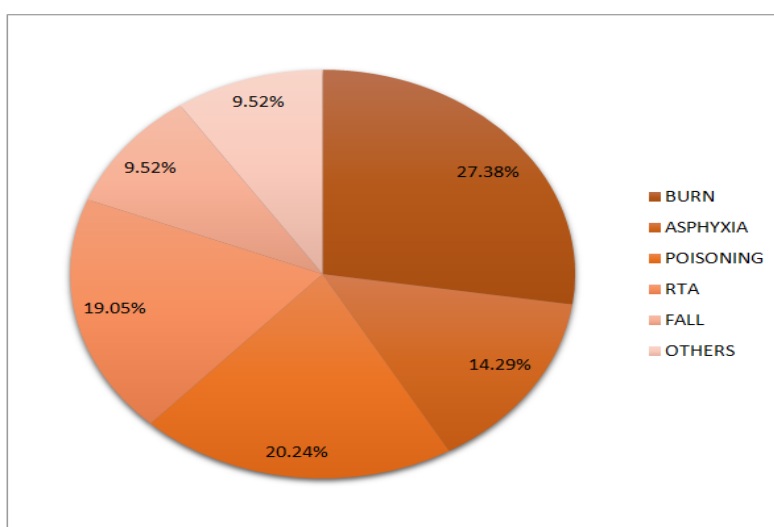


Diagrammatic Representation of Sex wise Distribution of cases.

➤ In the present study among total 84 cases causes of death were various. Number of burn cases were 23 i.e.27.38%. Total number of victims of asphyxia were 12 i.e.14.29% , where as the causes of asphyxia were hanging, drowning and strangulation. Among the poisoning cases various poisoning were alcohol poisoning, insecticide poisoning, copper sulphate poisoning, organophosphorus poisoning , snake bite and few unknown poisoning, where viscera send for chemical analysis and report being pending till date. Number of road traffic cases were 16,i.e.19.05%, number of fall from height were 8, i.e.9.52% and the others causes consist of Acute Myocardial Infarction and Brought Dead , number of cases were 8,i.e 9.52%.

Table – III: Distribution of cases depending on Cause of Death:

Cause Of Death	No Of Cases	% Of Cases
Burn	23	27.38
Asphyxia	12	14.29
Poisoning	17	20.24
Rta	16	19.05
Fall From Height	8	09.52
Others	8	09.52
Total	84	100.00



Diagrammatic Representation of Cases Depending on Cause of Death.

Table-IV: Correlation study of different parameters of Haemogram with Time Passed Since Death (Number of cases N-84)

	Hb	Hct	RBC	WBC	Platelet
TIME	-0.016	0.063	-0.454**	-0.335**	0.416**

** Correlation is significant at the 0.01 level (2-tailed).

- Value of Hemoglobin and Haematocrit in postmortem blood not correlated with time passed since death.
- Total number of RBC and WBC strongly correlated with Time passed since Death, though they are inversely correlated. It means with increase of time after death total number of RBC and WBC gradually decrease.
- Total number of Platelet also strongly correlated with Time passed since Death.

Table-V: Correlation study of different parameters of Haemogram with Time Passed Since Death depending upon cause of Death:

	Cause of Death	Hb	Hct	RBC	WBC	Platelet
TIME	Burn	0.002	-0.081	-0.512*	0.468*	0.374
	Asphyxia	0.271	0.152	-0.064	-0.313	0.372
	RTI	-0.230	-0.184	-0.519	-0.489	0.544
	Fall from Height	0.135	0.111	-0.292	0.910**	0.664
	Poisoning	-0.555	0.063	-0.501*	-0.264	0.515*

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

The above chart shows that total count of RBC and WBC has correlation with time passed since death in the victims of burn, fall from height and poisoning.

Total count of Platelet correlated with time passed since death in victims of poisoning.

VI. Discussion

In this present study total 84 cases were randomly selected depending upon the inclusion and exclusion criteria and their EDTA blood samples were drawn upto 48 hours of Death. The Haematological parameters from drawn EDTA blood sample were recorded through Automated analyser. The derived results were statistically analysed to calculate time passed since death.

In this study a total of 84 cases were selected from the corpses, among this 49 males and 35 females in the age group of 2.5 to 93 years. Out of these 84 cases 14(16.67%) were of 1 to 20 years age group, followed by 21 to 40 years age group, which was 39(46.43%), 41 to 60 years, which was 17(20.24%), 61 to 80 years, which was 12(14.28%) and 81 to 100 years which was 2(2.38%) . The sex distribution of the cases in the present study shows males are predominant than the females.

Here the study shows that the value of Haemoglobin and Haematocrit in postmortem blood not correlated with time passed since death. Professor J B Mukherjee in his book said that as fluid exudes out of vessels in dependent body parts in hypostasis, haematocrit value vary from place to place, hence not dependable.

The study also shows that total number of RBC and WBC strongly correlated with Time passed since Death, though they are inversely correlated. It means with increase of time after death total number of RBC and WBC gradually decrease.

In The Estimation of the Time Since Death in the Early Postmortem Period by Bernard Knight the loss of viability of white blood showed a moderate correlation with the postmortem period.

Rajesh Bardale showed lysis of Monocytes, Eosinophils, Neutrophils occur in 4 to 6 hours post-mortem interval. Lysis of Lymphocytes occurs in 8 to 10 hours interval. Lysis of RBC occur in 18 hours. Platelets become nonidentifiable in 21 hours postmortem interval.

Badapulle showed lysis of Eosinophils and Monocytes in 60 hours, Neutrophils in 66 hours and Lymphocyte in 84 hours. Whereas Dokgoz showed lysis of Eosinophils, Monocytes in 72 hours, Neutrophils in 96 hours, Lymphocytes in 120 hours post-mortem interval.

In this study total number of Platelet strongly correlated with Time passed since Death. This correlation is positive correlation that means upto 48 hours of death initial rise of total count of Platelets with time passed since death. H.Thomsen showed in his study that apparent initial increase in the platelet count in postmortem blood was found to be caused by hypostatic phenomena. The subsequent discontinuous decrease in the platelet count despite continuing hypostasis in the corpse can be explained in part by postmortem thrombolysis and the development of reversible platelet-platelet aggregates.

VII. Conclusion

It is told that a research often starts from an idea, a question in the mind of researcher or an extension of a previous line of enquiry. Without optimism most of the researches would never be started and completed. Studies of estimation of time passed since death was done by the various forensic experts. Their results also helped a lot. But the present study is not a mere extra addition to such previous researches, rather carries values because with the help of previous outcomes this present study could compare the similar aspect with the past and found some specific outcomes that is the reflection of this background only. Incorporation of computer software is a great turning point on this chapter. In Indian perspective such type of works are not so commonly carried out, but shows promising outcome. We know that research is systematic processes utilizing the scientific method for generating new knowledge that can be used to solve a problem to improve the existing status of a system. Research either for the academic interest or at least for the satisfaction of curiosity, has been seen to be useful in some way for the society in future days. In this aspect estimation of time passed since death is definitely useful. In a country like India, such type of works bear many difficulties, not always for the economic scarcity, but during the proceedings less cooperation from the legal guardian of the deceased. In medicolegal work the determination of the accurate time passed since Death has always been important. There is a continuous need for the development of an accurate method, by which the time of Death can be determined. For the last so many years the Blood has been considered as a rich source for this purpose.

In the present study the value of Hemoglobin and Haematocrit in postmortem blood is not found correlated with time passed since Death. In the present study total number of RBC and WBC strongly correlated with Time passed since Death, and are inversely correlated. It means with increase of time after death total number of RBC and WBC gradually decrease. Total number of Platelet also strongly correlated with Time passed since Death. When the cases are divided according to cause of Death, it was found that in the victims of Burn and Poisoning total count of RBC decrease with increase in time passed since Death. Total count of WBC

is strongly correlated with time since Death in the victims of Burn and very strongly in the victims of Fall from height. Total count of Platelet is correlated with time since Death in the victims of Poisoning.

VIII. Future Aspects

We can remember Claude Bernard “If an idea presents itself to us, we must not reject it simply, because it does not agree with the logical deduction of a reigning theory.” This essence is applicable for all research works. With the same essence and pursuit of exploration of commitment to truth, to the best of our belief and knowledge, this is a work of genuine stratagem in Indian scenario that can open a new horizon in the field of Forensic Medicine.

The study raise a demand that should be implemented in future that at every medical institute there should be provision of training on such topics which can be arranged in collaboration with Forensic Science department. Obstacles that may arise while conducting such works are various e.g., limited availability and accessibility to required resources, advanced scientific gazettes or vital literatures. From the present study it is inferred that estimation of total count of RBC, WBC and Platelet may provide an important data for estimation of the time passed since death. This present study reveals two aspects, one being the responsibility towards society and the other being responsibility to the academics to proceed on research works. Forensic Medicine is a stream where the scopes at both these aspects are sharply increasing with a demand to keep pace with the changing socio-economic structure. In view of this scenario, we can conclude that the present study is a step forward to fulfil the hope of society, satisfy the zeal of research and visualize the vistas of newer avenues.

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Abbreviation used

1. TSD- Time passed since death.
2. RTA-Road traffic accident.
3. OP Poisoning- Organophosphorus poisoning.
4. CuSO₄- Copper Sulphate
5. RBC- Red blood cells.
6. WBC-White blood cells.
7. Hb- Haemoglobin
8. Hct- Haematocrit

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