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ORIGINAL ARTICLE

Post-mortem peripheral smear: A forensic diagnostic tool?

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Abstract

The current study of post-mortem peripheral smears was conducted in Department of Forensic Medicine, Osmania Medical College with the main objective of studying the seasonal variability of autolysis of cell lines in peripheral circulation. In the study sample of 73 subjects, peripheral smears were made in the morgue immediately after collecting blood by venepuncture. Smears were stained with Leishman stain (A Romanowsky stain) within 2 hours of preparation and light microscope was used to study changes. The changes in cell morphology, membrane integrity, cytoplasmic and nuclear changes were studied in detail using valuable information from previous studies and conclusions were arrived at. At the outset white blood cells are most suitable for TSD predictability among others in peripheral smear because they withstand extraneous variables like refrigeration and show a similar degree of autolytic changes across all seasons. The validity of all such studies on cell lines for TSD was discussed from medico legal perspective. The take home message is all the microscopic changes studied in present study provided no added advantage significantly apart from traditional gross changes like rigor mortis, algor mortis, post-mortem lividity. If properly used on regular basis, algor mortis can enhance the quality of our reporting in India with respect to TSD.

Keywords

Post-mortem peripheral smear; Time since death; Microscopic forensics.

Introduction

The importance of estimating time since death has been appreciated for centuries.[1] It is the most vexatious question faced by a forensic pathologist all his life time. No single gross or microscopic post-mortem change can accurately predict the time elapsed after death. Each and every gross observation like algor mortis, rigor mortis, decomposition changes, microscopy and thantochemistry supplement each other in arriving at a predictable range of time since death. Review of literature shows good number of studies were done to evaluate cellular changes that occurred in blood after death to estimate time since death.[2-6] The aims and objectives of the study were to study changes in RBC, WBC and platelets with regard to time since death, to verify if studying microscopic changes of cell lines provide any advantage in complementing the gross changes in interpreting time since death, to study the pace of cellular changes in refrigerated and unrefrigerated cadavers and to study variability in pace of microscopic autolysis in summer and winter seasons.

A total of 73 cases in which time of death is known were studied as part of the study. 42 of these individuals were males, while 31 were females. The first phase of study was initiated in January 2018 during which 20 cases were studied. The subsequent phase of the study was conducted in May 2018 with a sample size of 53 cases. The sampling model followed is non-random purposive sampling.

The results obtained were used to predict time since death in 30 cases for which sampling was done randomly between January and May 2018. In these cases, our range of time elapsed since death was compared with the records to predict accuracy.

In 29 cases pre refrigeration and post refrigeration sampling was compared. It was not possible to compare the same in all cases due to receipt of bodies at odd hours and other cases being autopsied directly without being kept in cold storage.

Materials and Methods

Blood samples were collected from femoral vessels before autopsy. In case of failure of above method, blood was collected from common carotid artery, internal jugular vein or directly from heart using a 5cc syringe. Smears were prepared on the spot and stained using Leishman's stain. Light microscopy was used to study the prepared slides. The morphology of cells was studied and included the shape of cell, the cell membrane, cytoplasm and changes in the nucleus.[6]

Cases in which time since death was known precisely and within 48 hours of post-mortem interval were included in the study. Mutilated dead bodies, charred dead bodies, any cases with history of oncological, infectious or haematological diseases were excluded.

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Results

Demographic profile of the case and cause of death were not studied in detail during this study. However, care was taken to make the sample representative. The test population of 30 cases used for testing the results obtained in the study comprised of 22 males and 8 females. The cause of death for the population was asphyxia in 7, RTA in 12, poisoning in 6, and 5 had died due to other causes.

Table 1: Time intervals during which the respective changes in RBC are seen in summer and winter

RBC Cell Changes	Summer	Winter
Normal, no membrane or cytoplasmic changes	0-3 hours	0-5 hours
Dysmorphic RBC, central pallor intact and periphery red	3-12 hours	5-16 hours
Grossly dysmorphic RBC, central pallor reduced and periphery red	12-22 hours	12-24 hours
Grossly dysmorphic RBC, central pallor lost and periphery pale	20-31 hours	22-36 hours
Mixture of lysed and intact RBC, central pallor lost and periphery pale	24-36 hours	30-42 hours
Completely lysed RBC	20-48 hours	30-48 hours

Table 2: Time interval (in hours) during which the respective changes in WBC are seen in summer and winter

Cell type and autolytic changes	Summer	Winter
Neutrophils		
Pyknosis	6-12	6-12
Cytoplasmic & Nuclear vacuolation	12-18	12-18
Nuclear Fragmentation	16-24	16-24
Disintegration	>36	>48
Acidophils		
Pyknosis	6-12	6-12
Cytoplasmic & Nuclear vacuolation	12-18	12-18
Nuclear Fragmentation	16-24	16-24
Disintegration	>36	>48
Monocytes		
Pyknosis	6-12	6-12
Cytoplasmic & Nuclear vacuolation	12-18	12-18
Nuclear Fragmentation	>24-36	>24-36
Disintegration	>36	>36
Lymphocytes		
Nucleus Swollen, cytoplasm and cell membrane indistinct	>24	>24
Pyknosis	>36	>40
Nuclear Fragmentation	>48	>48
Disintegration	--	--

Table 3: Time interval during which the respective changes in platelets are seen in summer and winter

Platelets	Summer	Winter
Present	16-20 hours	16-22 hours
Absent	>16-20 hours	>16-22 hours

Table 4: positive predictability of results in the present study

Time range	Cases Studied	N	%
0-6 hours	5	4	80
6-12 hours	5	4	80
12-18 hours	5	3	60
18-24 hours	5	2	40
24-36 hours	5	1	20
36-48 hours	5	4	80

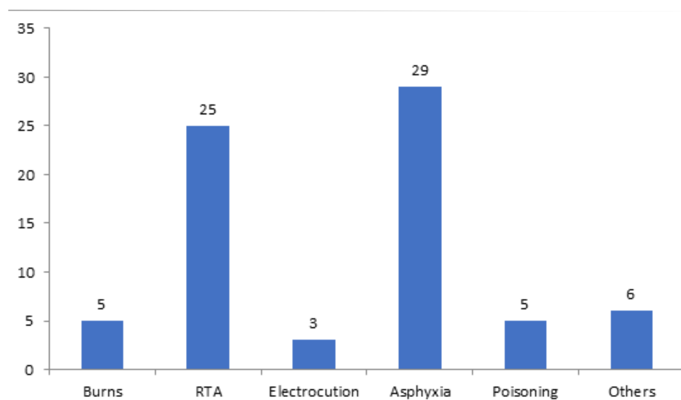

Figure 1: Cause of death profile for study population

In samples from refrigerated cadavers, RBC's discoid configuration transformed to echinocytic, spherocytic, and demonstrated crenation artefacts. However, the integrity of cell was preserved. WBC cell changes were stalled from further autolysis once they were refrigerated at 4 degrees centigrade in 22 cases and 7 cases showed further autolytic changes when smears were compared at autopsy. Increased fragility of WBC was demonstrated by presence of smudge cells in 8 cases along with disintegration of nuclear lobes and occasional nuclear budding and ragged cell membrane. It is difficult to opine whether they are storage artefacts or autolytic changes. Platelets showed no significant change pre and post refrigeration. Hence, it can be concluded that further autolysis of platelets is stalled by storing the cadaver at 4 degrees centigrade.

Because there was a wide variation in findings with respect to autolytic changes, we are presenting the autolytic changes seen in RBC (Table 1), WBC (Table 2) and platelets (Table 3) in time ranges. It can be inferred that the below mentioned changes in all cell lines were seen against the mentioned time period in our cross-sectional observation. A word of caution here is that they were not followed up during the range. Positive predictability of results is given in Table 4. The distribution of cases based on their cause of death is shown in Figure 1. Asphyxia was the leading cause of death, followed by road

traffic accidents.

The previous studies by Bardale et al.[6] and Kumar et al.[7] in this area were extremely helpful in the analysis of our data. The ranges in the study are upper and lower limits when the changes were noted first.

Discussion

The evidence required for legal medicine purposes in not just of indicative nature, it must be of probative standards. When the standard of proof required is beyond reasonable doubt to sustain the case fit for prosecution, the results of current study or previous studies conducted in this area of time since death is only of academic interest with almost no practical utility. It has been clearly established by this study that refrigeration of the corpse and seasonal variations cast a profound shadow of doubt in interpretation of results. The wide ranges of unpredictable changes in autolysis of cell lines makes standardization of data very difficult and hence further researchers are advised to desist from pursuing any research in this area. Peripheral smear cannot help in understanding the changes happening over lower ranges of time. Sub cellular and molecular forensics may provide some useful answers by studying over expression of some genes after death[8], degradation patterns of mRNA[9] etc.

In fact, a thorough research in molecular forensics is going to provide peculiar gene expression patterns that were actually silenced all during life and may provide new insights to understanding disease and evolution of the branch of personalized medicine.

When the results obtained in the study are used as a test for 30 cases the positive predictability of our results is shown in Table 4. No additional advantage is provided by these microscopic changes when compared to gross post-mortem changes in our study.

Conclusion

Future research in this area is strongly discouraged. With molecular autopsy making inroads in to Indian subcontinent, mRNA degradation-based methods and post-mortem gene activation-based methods are to be conducted in Indian scenario

Ethical clearance: A prior approval was obtained from the Institutional Ethics Committee

Conflict of interest: None to declare

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