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Original Article

Blood Cellular Changes Adjunct in Estimation of Time Since Death

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ABSTRACT

Background: Estimation of time since death (TSD) remains one of the most challenging aspects of forensic investigations. Conventional postmortem indicators such as rigor mortis, lividity, and body cooling provide only approximate estimates and are influenced by multiple environmental and physiological factors. Microscopic evaluation of postmortem blood changes has emerged as a potential adjunct method for refining early postmortem interval (PMI) estimation. Red blood cells (RBCs), owing to their structural characteristics and susceptibility to autolysis, undergo progressive morphological alterations after death that may correlate with increasing PMI.

Objective: The present study aimed to evaluate sequential morphological changes in RBCs in relation to time since death and assess their potential utility as microscopic indicators for estimating early and intermediate postmortem intervals.

Materials and Methods: This descriptive study was conducted over a period of 18 months in the Departments of Forensic Medicine & Toxicology and Pathology at Guru Gobind Singh Medical College and Hospital, Faridkot. A total of 100 medico-legal autopsy cases with documented hospital-certified time of death were included. Cases with hemolytic disorders, hematological malignancies, blood disorders, and charred bodies were excluded. After certification of death, bodies were stored at 4°C until autopsy. Cardiac blood samples were collected aseptically during autopsy, and thin peripheral blood smears were prepared, air-dried, and stained using Leishman's stain. Microscopic examination under oil immersion (100×) was performed to evaluate RBC morphology, including cell integrity, shape alterations, central pallor, and peripheral staining characteristics. Cases were categorized into seven TSD intervals ranging from 0–6 hours to beyond 48 hours. Data were recorded in a pre-designed proforma and analyzed using appropriate statistical methods.

Results: Progressive morphological alterations in RBCs were observed with increasing postmortem interval. RBC integrity remained preserved in all cases up to 12 hours and in the majority of cases up to 24 hours. Partial lysis became evident between 24–36 hours, with complete lysis observed beyond 36 hours. Morphological evaluation demonstrated a sequential transition from normal biconcave cells in the earliest interval to slight dysmorphism within 6–12 hours, followed by gross dysmorphism between 12–24 hours. Advanced degeneration and lysis predominated after 24 hours. Central pallor progressively diminished with increasing TSD, with intact pallor observed in early intervals and complete loss beyond 36 hours. Peripheral RBC appearance also demonstrated a transition from hemoglobinized margins to pale outlines and eventual loss of recognizable

cellular structures. These findings indicate a consistent and time-dependent pattern of postmortem RBC degeneration.
Conclusion: The study demonstrates that RBCs undergo predictable and sequential morphological changes following death. Preservation of intact morphology during the early postmortem interval, followed by progressive dysmorphism and eventual lysis, suggests that microscopic evaluation of RBCs can serve as a useful adjunctive tool in estimating time since death, particularly within the first 36–48 hours under controlled storage conditions. However, RBC morphology should be interpreted alongside other established postmortem indicators for more reliable estimation of the postmortem interval.

Keywords*:* Postmortem interval, Time since death, Red blood cells, RBC morphology, Postmortem blood changes, Forensic hematology, Medico-legal autopsy, Thanatology.

INTRODUCTION

Thanatology, the scientific study of death and its associated processes, encompasses the physiological, biochemical, and cellular events that occur following somatic death. The dying process follows a sequential progression beginning with clinical death, advancing to brain death, followed by biological death, and culminating in cellular death. Brain death rapidly ensues due to oxygen deprivation, initially affecting the cerebral cortex, subsequently the cerebellum, and finally the lower brain centers. Once the brainstem and vital autonomic centers cease functioning, irreversible cellular death occurs.

Following death, a cascade of physico-chemical changes takes place in a relatively predictable sequence. These include algor mortis, rigor mortis, livor mortis (hypostasis), and ultimately decomposition. Early observable changes such as corneal opacity and loss of corneal firmness occur irrespective of eyelid position. Collectively, these postmortem alterations form the basis for estimating the Time Since Death (TSD), a critical component of forensic investigations.

Estimation of TSD is of paramount importance in medicolegal practice. Forensic experts are frequently required to provide scientifically grounded opinions regarding the postmortem interval (PMI) in courts of law. Although such estimations are inherently subject to variability and cannot yield absolute precision, they remain an essential responsibility of the autopsy surgeon. A comprehensive assessment incorporating multiple postmortem indicators is necessary to establish a reasonable time range. In contemporary judicial systems, courts increasingly rely on scientific evidence to substantiate investigative findings and ensure evidentiary reliability (1).

Accurate determination of TSD not only assists in establishing identity but also plays a decisive role in criminal investigations, particularly in homicide cases, where narrowing the temporal window can significantly aid in suspect identification. Despite advancements in forensic methodologies, the demand for more accurate and reproducible techniques for PMI estimation persists, especially as the postmortem interval lengthens (2).

The fundamental principle underlying TSD estimation involves the analysis of measurable biological or physical parameters that exhibit time-dependent progression following death. These parameters can be conceptualized as forming a progression curve originating at the time of death. The configuration, slope, and initial point of this curve are influenced by numerous intrinsic and extrinsic variables, including environmental temperature, humidity, pre-mortem physiological status, and postmortem conditions. wingto the influence of these variables, TSD estimation is typically expressed as a time range rather than a specific moment(3).

Over the past several decades, biochemical techniques have been increasingly explored to enhance PMI estimation. These methods involve analysis of body fluids such as blood, cerebrospinal fluid, aqueous humor, and vitreous humor, all of which undergo systematic chemical changes following death. Each biochemical marker demonstrates a distinct rate and pattern of alteration, potentially providing valuable temporal indicators (4).

In addition to classical and biochemical methods, PMI estimation may incorporate forensic entomology, evaluation of gastrointestinal and urinary contents, and biochemical analysis of various body fluids including pericardial fluid and bone marrow. Circumstantial evidence such as hair changes, lice infestation, clothing condition, and personal effects may also contribute to narrowing the estimated interval.

Extensive research has focused on postmortem alterations in blood. Parameters including pH, hematocrit, electrolytes, and enzymatic levels have been studied in relation to TSD. While certain components remain relatively stable, others exhibit either predictable or variable changes over time (5).

Red blood cells (RBCs), or erythrocytes, are anucleate biconcave cells derived from hematopoietic stem cells in the bone marrow. They facilitate oxygen and carbon dioxide transport via hemoglobin, whose iron-containing heme component imparts the characteristic red coloration of blood (6).

Review of literature

Penttilä and Laiho (1980) conducted one of the earliest systematic evaluations of postmortem peripheral blood morphology in 123 medicolegal autopsies with PMIs ranging from 1.7 to 270.4 hours. Stored at 4°C, samples demonstrated progressive transformation of RBCs intoechinocytes and spherocytes (7).

Babapulle and Jayasundera (1990) observed progressive decline in identifiable blood cells between 36 and 84 hours postmortem. Notably, morphological differences between in vivo postmortem and in vitro stored samples became evident after 36 hours, underscoring methodological variability (8).

Bardale and Dixit (2007), in a study of 80 autopsy cases, reported that RBCs remained identifiable up to 18 hours and platelets up to 20 hours (9).
Shah et al. (2015) further demonstrated that intact RBCs up to 19 hours allowed reliable postmortem blood grouping using tube agglutination techniques (10).

Kundu et al. (2017) found strong inverse correlations between RBC counts and PMI in 84 cases, although hemoglobin and hematocrit values showed limited correlation (5).
Jat et al. (2019) confirmed time-dependent RBC lysis after 24 hours (11).
Tyagi et al. (2020) demonstrated consistent morphological changes in RBCs and WBCs but concluded that routine hemogram indices lack reliability as standalone PMI markers (12).
Anand et al. (2021), in a large series of 210 cases, observed initial RBC crenation at four hours and complete lysis by approximately eleven hours, highlighting the potential value of early morphological transitions (13).

MATERIALSANDMETHODS

This descriptive study was conducted over a period of 18 months in the Departments of Forensic Medicine & Toxicology and Pathology at Guru Gobind Singh Medical College and Hospital, Faridkot. A total of 100 consecutive hospital deaths with documented and certified time of death were included using a non-random convenient sampling technique after obtaining written informed consent from the relatives or guardians. Cases with known hemolytic disorders, hematological malignancies, other blood disorders, and charred bodies were excluded. After certification of death, all bodies were stored at 4°C until autopsy, and the recorded hospital time of death was considered the reference for calculating time since death (TSD). Cases were categorized into seven groups based on TSD for analytical purposes. During medico-legal autopsy, approximately 10 mL of cardiac blood was collected aseptically, properly labeled, and used to prepare thin peripheral blood smears, which were air-dried and stained using Leishman's stain. Microscopic examination under oil immersion (100×) was performed to evaluate sequential morphological changes in red blood cells and white blood cells, including alterations in shape, central pallor, membrane integrity, nuclear changes, cytoplasmic vacuolation, and lytic activity. All findings, along with postmortem details, cause, and manner of death, were recorded in a pre-designed proforma. The collected data were entered into Microsoft Excel and analyzed using appropriate statistical tests to assess correlations between hematological changes and TSD, while maintaining ethical standards and confidentiality throughout the study.

RESULTS

The present study included 100 medico-legal autopsy cases with documented hospital-certified time of death. Cases were stratified into seven time-since-death (TSD) intervals: 0–6 hours, 6–12 hours, 12–18 hours, 18–24 hours, 24–36 hours, 36–48 hours, and >48 hours. Cardiac blood samples were examined microscopically to assess sequential postmortem morphological alterations in red blood cells (RBCs).

The age of the deceased ranged from 7 months to 72 years (mean 38.28 ± 13.89 years), with the majority belonging to the 31–40-year age group (38%). Males constituted 81% of cases. The most common alleged cause of death was road traffic accidents (58%), followed by poisoning (31%). Most autopsies were conducted between 12 and 24 hours postmortem (49%), followed by 24–36 hours (23%) as shown in Table 1.

Table 1: Age-wise distribution of deceased individuals

Age Group (in years)	No. of Deceased Individuals	Percentage
0-10 Years	1	1%
11-20 Years	6	6%
21-30 Years	22	22%
31-40 Years	38	38%
41-50 Years	13	13%
51-60 Years	12	12%

61-70 Years	7	7%
≥71 Years	1	1%
Total	100	100%
Mean±SD	**38.28±13.89**	

In this study, male subjects constituted the majority of cases, accounting for 81% of the sample, while females comprised of 19% cases. This marked male predominance as there is higher exposure of males to medico-legal circumstances requiring autopsy examination as shown in Table 2.

Table 2: Gender-wise distribution of deceased individuals

Gender	No. of Deceased Individuals	Percentage
Male	81	81%
Female	19	19%
Total	**100**	**100%**

The most common alleged cause of death in the present study was road traffic accidents, accounting for 58% of cases, followed by poisoning in 31% of cases. The remaining 11% were attributed to various other causes. This distribution highlights the significant contribution of trauma and toxicological factors in medico-legal autopsy cases as shown in Table 3.

Table 3: Alleged cause of death of deceased individuals

Alleged Cause of Death	No. of Deceased Individuals	Percentage
Road Traffic Accident	58	58%
Poisoning	31	31%
Others	11	11%
Total	**100**	**100%**

The time passed since death among the studied cases ranged from less than 6 hours to over 48 hours. The majority of cases i.e. 49% were observed within 12–24 hours after death, with 24% between 12–18 hours after death and 25% between 18–24 hours respectively. Cases within 24–36 hours accounted for 23%, while only a small proportion were seen in the extremes—1% cases within 0–6 hours and 6% cases beyond 48 hours. This distribution reflects the practical constraints and typical delays in conducting medico-legal autopsies as shown in Table 4.

Table 4: Time passed since death before commencement of autopsy

Time Passed Since Death	No. of Deceased Individuals	Percentage
0-6 Hours	**1**	**1%**
6-12 Hours	**14**	**14%**
12-18 Hours	**24**	**24%**
18-24 Hours	**25**	**25%**
24-36 Hours	**23**	**23%**
36-48 Hours	**7**	**7%**
>48 Hours	**6**	**6%**
Total	**100**	**100%**

RBC Integrity and Lysis

Assessment of RBC integrity demonstrated that 72% of cases exhibited intact RBCs, while 28% showed varying degrees of lysis. A clear temporal association was observed between increasing TSD and RBC degeneration. In the early postmortem interval (0–6 hours), RBCs were intact in 100% of cases. Similarly, complete preservation was observed up to 12 hours. Between 12 and 18 hours, 95.8% of samples retained intact RBCs. Integrity remained preserved in all cases within the 18–24-hour interval.

A marked transition occurred beyond 24 hours. In the 24–36-hour group, 60.8% of cases exhibited RBC lysis. Complete lysis was observed in all cases beyond 36 hours (36–48 hours and >48 hours). These findings demonstrate a progressive and time-dependent pattern of RBC membrane disruption, with a critical threshold occurring after 24 hours postmortem.

Table 5: Distribution on the basis of integrity of RBCs at different time intervals.

TSD	Intact	Lysed	Total
0-6 Hours	**1(100%)**	**0**	**1**
6-12 Hours	**14(100%)**	**0**	**14**
12-18 Hours	**23(95.8%)**	**1(4.1%)**	**24**

18-24 Hours	**25(100%)**	**0**	**25**
24-36 Hours	**9(39.1%)**	**14(60.8%)**	**23**
36-48 Hours	**0**	**7(100%)**	**7**
>48 Hours	**0**	**6(100%)**	**6**

Morphological Alterations in RBC Shape

Evaluation of RBC morphology revealed a sequential progression from normal biconcave forms to dysmorphism and eventual lysis. Overall, only 2% of cases retained normal RBC morphology. Slight dysmorphism was observed in 16%, gross dysmorphism in 57%, and complete lysis in 25% of cases.

Within 0–6 hours, RBCs retained normal morphology in all cases. In the 6–12-hour interval, 57.1% exhibited slight dysmorphism, while 35.7% demonstrated gross dysmorphic changes. Gross dysmorphism became the predominant pattern between 12–18 hours (75%) and increased further between 18–24 hours (92%).

Lytic changes became prominent in the 24–36-hour interval (56.5%), indicating advanced membrane disintegration. This proportion increased to 85.7% in the 36–48-hour group, with 100% lysis observed beyond 48 hours. The findings reflect a consistent and ordered progression of postmortem RBC degeneration, correlating strongly with increasing TSD.

Table 6: Distribution on the basis of shape of RBCs at different time intervals

TSD	Normal	Slightly Dysmorphic	Grossly Dysmorphic	Lysed	Total
0-6 Hours	1(100%)	0	0	0	1
6-12 Hours	1(7.1%)	8(57.1%)	5(35.7%)	0	14
12-18 Hours	0	6(25%)	18(75%)	0	24
18-24 Hours	0	2(8%)	23(92%)	0	25
24-36 Hours	0	0	10(43.4%)	13(56.5%)	23
36-48 Hours	0	0	1(14.2%)	6(85.7%)	7
>48 Hours	0	0	0	6(100%)	6

Changes in Central Pallor

Central pallor, reflecting RBC membrane integrity and hemoglobin distribution, demonstrated progressive loss with increasing postmortem interval. Overall, intact central pallor was observed in 10% of cases, reduced pallor in 41%, and complete loss in 49%.

In the earliest interval (0–6 hours), central pallor was preserved in all cases. By 6–12 hours, only 50% retained intact pallor. Between 12–18 hours, 75% showed reduced central pallor, while 20.8% demonstrated complete loss. In the 18–24-hour interval, 72% exhibited complete loss of central pallor.

Beyond 36 hours, central pallor was absent in all cases. This consistent decline suggests that redistribution of hemoglobin and membrane structural breakdown occur progressively and may serve as reliable microscopic indicators of advancing postmortem interval.

Table 7: Distribution on the basis of central pallor of RBCs at different time intervals

TSD	Intact	Reduced	Lost	Total
0-6 Hours	**1(100%)**	**0**	**0**	**1**
6-12 Hours	**7(50%)**	**7(50%)**	**0**	**14**
12-18 Hours	**1(4.1%)**	**18(75%)**	**5(20.8%)**	**24**
18-24 Hours	**1(4%)**	**6(24%)**	**18(72%)**	**25**
24-36 Hours	**0**	**10(43.4%)**	**13(56.5%)**	**23**
36-48 Hours	**0**	**0**	**7(100%)**	**7**
>48 Hours	**0**	**0**	**6(100%)**	**6**

Peripheral Appearance of RBCs

Assessment of RBC peripheral staining characteristics further supported the temporal pattern of degeneration. Overall, 19% of cases exhibited a hemoglobinized periphery, 62% showed a pale periphery, and 19% were unrecognizable due to advanced lysis.

All cases within 0–6 hours demonstrated hemoglobinized peripheral margins. In the 6–12-hour group, 78.5% retained hemoglobinized periphery, while 21.4% appeared pale. A marked shift toward peripheral pallor was observed in the 12–18-hour interval (83.3% pale). Between 18–24 hours, 84% showed pale periphery and 4% were unrecognizable.

From 24–36 hours onward, hemoglobinized cells were no longer observed. The proportion of unrecognizable cells increased progressively, reaching 100% beyond 48 hours. These changes reflect progressive hemoglobin diffusion and membrane degradation associated with autolytic processes.

Table 8: Distribution on the basis of periphery of RBCs at different time intervals.

TSD	Hemoglobinized	Pale	Not Recognized	Total
0-6 Hours	1(100%)	0	0	1
6-12 Hours	11(78.5%)	3(21.4%)	0	14
12-18 Hours	4(16.6%)	20(83.3%)	0	24
18-24 Hours	3(12%)	21(84%)	1(4%)	25
24-36 Hours	0	17(73.9%)	6(26.1%)	23
36-48 Hours	0	1(14.3%)	6(85.7%)	7
>48 Hours	0	0	6(100%)	6

Overall Pattern

Collectively, the findings demonstrate a reproducible and sequential pattern of RBC degeneration characterized by (i) preservation of morphology and central pallor in early postmortem intervals (<12 hours), (ii) progressive dysmorphism and pallor reduction between 12–24 hours, and (iii) marked lysis and structural disintegration beyond 24–36 hours. Complete lysis was consistently observed after 36 hours.

The temporal consistency of these morphological alterations suggests that microscopic evaluation of RBCs may serve as a useful adjunctive tool in estimating early and intermediate postmortem intervals under controlled storage conditions.

DISCUSSION

In the present study, the highest proportion of deceased individuals was 38 cases i.e 38% belonged to the age group of 31 to 40 years.

Comparatively, in a study conducted by Jat SS. et al. (2019), the majority of cases i.e. 27.33% were from the 21 to 30 years age group, followed closely by individuals aged 31 to 40 years i.e. 26.66%(11).

Similarly, Shah K. et al. (2015) reported that out of 29 total cases, 75% of the individuals were between the ages of 21 and 50 years(10).

In a broader demographic range, Anand A. et al. (2021) analyzed 210 cases involving individuals aged between 18 and 76 years(13).

Meanwhile, Kundu S Das and Dutta SS (2017) examined 84 cases and observed that the majority i.e. 46.43% were within the 21 to 40 years age group, followed by 20.24% in the 41–60 years bracket(5).

These findings collectively suggest that the 21 to 40 years of age range is commonly affected across multiple studies, likely reflecting increased exposure to risk factors such as accidents, occupational hazards and lifestyle-related mortalities among other causes of death prevalent in this age group.

In the present study, a marked gender disparity was observed in hospital deaths, with males accounting for 81% of the total cases and females comprising only 19%.

This predominance of male subjects aligns with the findings reported by Jat SS et al. (2019), who documented a male-to-female ratio of 4.35:1 in his study(11).

Similar trends were noted in the study conducted by Bardale R and Dixit PG, (2007) which involved of total 80 cases with 60 males and 20 females, further supporting the male predominance in medico-legal autopsies(9).

Likewise, the study by Kundu S Das and Dutta SS (2017) included total 84 cases, of which 49 were males and 35 were females, again reflecting a higher incidence in males(5).

In contrast, the study by Shah K et al. (2015), which comprised 29 cases with 14 males and 15 females, demonstrated an almost equal gender distribution, which diverges from the trend observed in our study(10).

The consistent male predominance across most studies may be attributed to higher exposure of males to road traffic accidents, occupational hazards and risk-taking behavior, which potentially increases their vulnerability to unnatural deaths requiring forensic examination.

In the present study, the predominant alleged cause of death was road traffic accidents (RTAs), accounting for 58% cases, followed by poisoning in 31% cases, and 11% cases attributed to other causes. This distribution highlights the significant contribution of accidental and unnatural deaths to medico-legal autopsy cases.

In comparison, Kundu S Das and Dutta SS(2017) observed a different pattern in their study of 84 cases, where burn injuries constituted the largest group with 27.38% of cases, followed by asphyxial deaths, including hanging, drowning, and strangulation, comprising of 14.29% cases. Poisoning cases in their study were diverse, including alcohol, insecticide, copper sulfate, organophosphorus compounds, snake bites, and a few of undetermined nature pending chemical analysis. RTAs in their study accounted for 19.05%, which is substantially lower than in the present study. Additionally, fall from height in 9.52% cases and other causes such as acute myocardial infarction and cases brought dead in 9.52% cases were also reported. The variation in the cause-of-death distribution between the two studies may be influenced by regional differences in trauma incidence, access to healthcare, urbanization, and patterns of substance use. The predominance of

RTA cases in the current study underscores the ongoing public health burden of traffic-related fatalities and their significant representation in forensic practice(5).
In the present study, the postmortem interval (PMI) ranged from 0 to 48 hours, with the highest number of cases i.e. 25% observed in the 18–24-hour interval, followed closely by 24% cases in the 12–18-hour range. The least number of cases i.e. 1% was recorded in the 0 to 6 hour interval, and 6% cases exceeded the 48 hour mark.
These findings are comparable to those reported by Jat SS et al. (2019), where the majority of cases fell within the 12–18-hour and >48-hour intervals, each constituting 22% of their total sample(11).
Studies by Penttilä A. and Laiho K. (1981) extended their observation over a wide PMI range from 1.7 to 270.4 hours, reflecting an extensive temporal scope(7).
Similarly, Babapulle C.J. and Jayasundera N.P. (1993) examined changes in corpses over a period of 0 to 84 hours(8).
In contrast, Bardale R and Dixit PG (2007) limited their observations to a 24-hour window, likely due to the study being conducted on non-refrigerated cadavers, which may accelerate postmortem changes(9).
Shah K et al. (2015) focused on a narrower PMI range of 2.5 to 19 hours. The variation in observation periods across studies reflects differences in study design, refrigeration conditions, and practical feasibility(10).
In the present study, the morphological integrity of RBCs was observed to degrade progressively with increasing postmortem interval (PMI). Up to 24 hours after death, all cases demonstrated complete RBC integrity, indicating that cellular structures remained intact in early postmortem periods. In the 24–36 hour group, a transition was noted, with 14 out of 23 cases (60.8%) showing complete lysis, while the remaining cases still displayed intact cells. Beyond 36 hours, RBCs were completely lysed in all examined cases (7/7 in the 36–48 hour group and 6/6 in the >48 hour group), indicating advanced autolysis.
These findings are in strong agreement with the study conducted by Jat SS et al. (2019), who observed complete integrity of RBCs up to 24 hours postmortem. Their study similarly reported partial lysis between 24 and 36 hours, and complete lysis thereafter(11).
Kumar B et al. (2015) also reported that 94.7% of RBCs remained intact up to 18 hours, with progressive lysis occurring between 18–48 hours and no intact cells beyond 48 hours. Our observation of one early case showing lysis in the 12–18 hour group (1/24) may reflect individual variability due to environmental factors, body storage conditions, or cause of death(14).
Anand A et al. (2021) further supported the trend of early morphological changes, reporting crenated RBC margins as early as 4 hours and complete lysis beginning by 11 hours in some cases. Although their timeline appears more accelerated, the overall progression remains consistent(13).
Temperature may play a critical role, as highlighted in the study by Penttilä and Laiho (1981), which demonstrated that RBCs retained structural integrity for longer durations at +4°C. This aligns with our study, where refrigeration was maintained, likely contributing to delayed lysis(19).
Collectively, these findings highlight the diagnostic potential of evaluating RBC integrity as a time-sensitive morphological marker in postmortem analysis. The preservation of RBCs within the first 24 hours, followed by a phase of partial to complete lysis between 24 to 48 hours, supports its application in estimating the postmortem interval during early decomposition stages.

In the present study, red blood cell morphology showed progressive distortion with increasing postmortem interval (PMI). In the initial time range of 0–6 hours, the single observed case (1/1; 100%) exhibited normal RBC shape. In the 6–12 hour interval, 7.1% (1/14) of cases showed normal shape, 57.1% (8/14) were slightly dysmorphic, and 35.7% (5/14) were grossly dysmorphic. Between 12–18 hours, 25% (6/24) were slightly dysmorphic while 75% (18/24) had grossly dysmorphic cells. In the 18–24 hour group, the majority of cases, 92% (23/25), were grossly dysmorphic, with 8% (2/25) being slightly dysmorphic. From 24–36 hours, 43.4% (10/23) of cases had grossly dysmorphic RBCs and 56.5% (13/23) showed complete lysis. Beyond 36 hours, nearly all cases exhibited lysis: in the 36–48 hour group, 1 case (14.3%) showed gross dysmorphism while 6 cases (85.7%) were lysed; and in the >48 hour group 100% (6/6) of RBCs was lysed.

These observations are closely aligned with the findings of Kumar B et al. (2015). In their study, within the first 6 hours after death, 38.9% of cases exhibited normal RBC morphology, while 61.1% were slightly dysmorphic. Between 6–12 hours, only 7.1% retained normal shape, 60.7% were slightly dysmorphic, and 32.2% were grossly dysmorphic. These findings are comparable to our results for the same period (7.1% normal, 57.1% slightly dysmorphic, 35.7% grossly dysmorphic). In the 12–18 hour interval, Kumar et al. reported 87.5% grossly dysmorphic cells, closely matching the 75% observed in our study. Their study showed 100% grossly dysmorphic RBCs in the 18–24 hour period, mirroring the 92% in our study. In the 24–36 hour group, Kumar et al. found 55.6% showed gross dysmorphism, 11.1% showed mixture of dysmorphic and lysed cells, and 33.3% showed complete lysis, which is in line with our observation of 43.4% gross dysmorphism and 56.5% showed complete lysis. Complete lysis in all cases beyond 48 hours was reported by both studies(14).

SS Jat et al. (2019) reported that RBCs became slightly dysmorphic between 6–12 hours and grossly dysmorphic after 12–24 hours. Beyond 24 hours, a mixture of grossly dysmorphic and microcytic cells was seen, with complete lysis occurring after 36 hours. These findings are consistent with our observations, particularly the clear transition from dysmorphic to

lysed morphology between 24 and 48 hours(11).

Penttilä and Laiho (1981) noted that most RBCs were quite normal or slightly crumbled up to 12 hours. Between 12–48 hours, disc-shaped forms were predominant, and beyond 48 hours, crenated or speculated cells dominated. Though their terminology and morphological grading differ slightly, the progression of distortion correlates with our findings of increasing dysmorphism and eventual lysis(7).

Shah K et al. (2015) identified morphological alterations in 37% of cases, noting that these changes were more closely associated with the cause of death than with the PMI. Among the altered cells, 4 cases showed crenation, all associated with mechanical trauma and IV fluid administration. While their study emphasized the role of external factors, our findings suggest a stronger correlation between dysmorphism and increasing PMI, though it is important to consider that factors such as trauma and medical intervention may also influence cellular morphology(10).

Overall, the shape of RBCs in our study changed progressively with TSD. Slight dysmorphism appeared by 6–12 hours after death, gross dysmorphism became dominant between 12–24 hours, and cell lysis was seen in most cases after 24–36 hours. These results are in broad agreement with previous studies by Kumar B et al., Jat SS et al., and others, reinforcing that morphological assessment of RBC shape offers valuable information in estimating the postmortem interval, particularly within the first 48 hours after death.

In the present study, the central pallor of RBCs exhibited a progressive deterioration with increasing postmortem interval (PMI). In the earliest time bracket of 0–6 hours, all cells (1/1; 100%) retained a normal central pallor. Between 6–12 hours, central pallor was intact in 50% (7/14), while the remaining 50% (7/14) showed a reduction. From 12–18 hours, only 4.1% (1/24) retained central pallor, 75% (18/24) showed reduction, and 20.8% (5/24) showed complete loss. Between 18–24 hours, loss of central pallor became prominent in 72% (18/25) of the cases, with only 4% (1/25) retaining intact pallor and 24% (6/25) showing reduced pallor. In the 24–36 hour group, 43.4% (10/23) had reduced central pallor, while 56.5% (13/23) showed complete loss. Beyond 36 hours, loss of central pallor was universal—100% of RBCs had lost pallor in all 7 cases examined in the 36–48 hour group, and in all 6 cases examined beyond 48 hours.

These findings correlate well with those of Kumar B et al. (2015), who also reported a progressive loss of central pallor with time. In their study, 81.8% of RBCs retained normal central pallor within the first 6 hours, aligning with the 100% intact cases in our own 0–6 hour group. Between 6–12 hours, they found reduced pallor in 82.1% of cases and a small percentage of cases 3.6% with complete loss, compared to our 50% reduced pallor and no loss. A sharp increase in loss was noted in both studies between 12–24 hours. In Kumar's study, 37.5% of cases between 12–18 hours and 79.4% between 18–24 hours had lost central pallor, closely matching our own results of 20.8% and 72% respectively for the same intervals. Both studies further reported complete loss and lysis of cells after 36 hours, indicating a consistent degradation timeline(14).

Jat SS et al. (2019) also described a similar pattern. They observed retention of central pallor up to 6 hours, reduction between 6–18 hours, and complete loss after 18 hours—which was consistent with findings of the present study, particularly the near-complete loss seen by 24 hours after death(11).
While Shah K et al. (2015) did not quantify the central pallor across time intervals in the same detailed manner; they reported absence of central pallor in a subset of 7 out of 11 cases showing abnormal cell morphology. Although their observations were more qualitative and related partly to causes of death, they still support the trend of postmortem morphological deterioration including loss of pallor(10).
Overall, the present study reinforces the consistent and predictable progression of central pallor changes in RBCs following death. Our findings are in agreement with those of Kumar B et al., Jat SS et al., and Shah K et al., demonstrating that central pallor is a sensitive morphological parameter that begins to diminish as early as 6 hours postmortem and are typically lost beyond 24 hours, with complete lysis apparent after 36–48 hours. These observations highlight the potential utility of central pallor evaluation as a microscopic marker for estimating the postmortem interval, particularly within the first 36 hours.
In the present study, the morphology of the RBC periphery showed a clear pattern of degradation with increasing postmortem interval (PMI). At 0–6 hours, all RBCs (1/1; 100%) exhibited hemoglobinized peripheries, indicating intact cellular outlines. Between 6–12 hours, 78.5% (11/14) of RBCs retained hemoglobinized peripheries, while 21.4% (3/14) of RBCs began to appear pale. A marked shift was noted from 12–18 hours, where only 16.6% (4/24) of RBCs retained hemoglobinized peripheries, and 83.3% (20/24) of RBCs were pale. In the 18–24 hour interval, 84% (21/25) of cells had pale peripheries while 12% (3/25) of cells retained hemoglobinization, and 4% (1/25) cells were no longer morphologically recognizable. This trend of degradation intensified in the 24–36 hour group, where 73.9% (17/23) cells were pale and 26.1% (6/23) cells were completely unrecognizable. Beyond 36 hours, peripheral disintegration was nearly complete. 85.7% (6/7) of RBCs were unrecognizable in 36–48-hour group, and in all 6 cases examined beyond 48 hours, 100% of cells had lost peripheral structure entirely.

These findings are in strong agreement with those of Kumar B et al. (2015), who also observed progressive peripheral morphological changes with TSD. In their study, 90.9% of RBCs retained a red (hemoglobinized) periphery within the first 6 hours, closely aligning with the 100% observed in our 0–6-hour group. Between 6–12 hours, they reported 64.3% hemoglobinized and 35.7% pale peripheries, comparable to our 78.5% and 21.4% respectively. A sharp decline was noted in both studies between 12–18 hours, with only 7.5% of cells retaining red peripheries in Kumar's study, compared to 16.6% in the present study. By the 18–24-hour period, 100% of cells had pale peripheries in Kumar's study, consistent with the present study with 84% pale and 4% unrecognizable findings. Furthermore, both studies showed increased lysis and loss of peripheral integrity beyond 24 hours, with Kumar reporting 83.3% lysis at 36–48 hours, while the present study noted 85.7% unrecognizable peripheries in the same interval(14).

Jat SS et al. (2019) also documented a similar degradation pattern. Hemoglobinized peripheries were observed up to 18 hours, followed by pallor from 18–36 hours, and complete unrecognizability beyond 36 hours. This timeline mirrors our findings and further supports the notion of peripheral changes as a reliable marker of postmortem interval. Despite minor inter-study variations, likely attributable to case demographics, environmental factors, and slide processing techniques, the overall trend remains consistent(11).

Thus, the present study corroborates the temporal sequence of peripheral RBC changes reported by Kumar B et al. and Jat SS et al., reinforcing the diagnostic value of peripheral RBC morphology in forensic investigations. The gradual loss of hemoglobinized outline and transition to pallor and eventual unrecognizability offers a practical microscopic parameter for estimating the postmortem interval, particularly in the early to intermediate postmortem stages up to 48 hours.

CONCLUSION

The present study demonstrates that red blood cells (RBCs) undergo a consistent and sequential pattern of morphological alterations following death, even under controlled refrigerated conditions. Preservation of normal morphology and central pallor was observed within the early postmortem interval, particularly during the first 12 hours. Progressive dysmorphism became prominent between 12 and 24 hours, followed by significant membrane disruption and lysis after 24 hours. Complete RBC disintegration was uniformly observed beyond 36 hours.

These findings indicate that RBC degeneration follows an ordered autolytic progression rather than abrupt structural collapse, suggesting its potential applicability as a supplementary microscopic parameter in estimating early and intermediate postmortem intervals. The predictable transition from intact cells to dysmorphism and eventual lysis may assist forensic practitioners in narrowing the time since death, particularly when interpreted alongside conventional postmortem changes and investigative findings.

Although RBC morphology should not be considered a standalone determinant of postmortem interval, its reproducible time-dependent alterations support its value as an adjunctive tool in forensic practice. Further studies incorporating larger sample sizes, environmental variability, and quantitative morphometric techniques may enhance the reliability and standardization of this approach in medico-legal investigations.

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