A Study on Postmortem Wound Dating by Gross and Histopathological Examination of Abrasions

Javaregowda Vinay, MD,* Sathyanarayana Harish, MD,† Gouri S.R. Mangala, MD,‡ and Basappa S. Hugar, MD, DNB†

Introduction: Abrasions are the most common blunt force injuries. The precise dating of injury is extremely important in forensic medicine practice. As we know, the wound healing occurs in well-orchestrated sequence, consisting of inflammation, proliferation, and maturation.

A study of occurrence of such phases will help in understanding the sequence of events in wound healing. In this context, this study of wound dating from gross and microscopic level was taken.

Materials and Methods: Postmortem study of wound dating by gross and histopathological examination of abrasions was carried out in the Department of Forensic Medicine, in M.S. Ramaiah Medical College. A total of 101 abrasions were correlated to time frame the occurrence of different gross changes and microscopic changes that follow the blunt trauma. Abrasions ranging from 0 hour to a maximum of 45 days were studied.

Results: The gross changes of abrasions were in correlation with the microscopic changes; however, the role of the comorbid conditions is significant because the results showed variations with respect to healing process. **Conclusions:** This study signifies that, if naked eye examination is studied along with histopathological examination, the reliability and accuracy of dating of wound increase. Whenever accurate determination of age is required, the autopsy surgeon can subject the samples for histopathological examination and correlate before opining the age of injury.

Key Words: gross changes, histopathology, dating of abrasions

(Am J Forensic Med Pathol 2017;38: 167-173)

wound or injury is defined as damage to any part of the body A due to the application of mechanical force. The term wound describes the morphological and functional disruption of the continuity of a tissue structure. Mechanical injuries may be due to blunt force, sharp force, or firearms. Deaths due to blunt force trauma resulting from road traffic accidents, fall from height, assault, and so forth are the most common cases encountered by the forensic experts. Where in the type of wound, site, size, pattern, and nature of the wound as to antemortem or postmortem has to be ascertained, apart from this, the age of the wound has significance in forensic pathology. To establish the causal relationship between times of infliction to the time of death, the age of the wound has to be determined more accurately in the crime investigation to fix the responsibility. The precise dating of injury is extremely important even in clinical forensic medicine. Forensic experts are frequently asked to comment on the age of injuries in the court, and the interpretation may have significant medicolegal consequences.² In such situations, reliability of interpretation becomes of prime importance. Blunt force injuries are encountered in both living and dead, which

Manuscript received September 13, 2016; accepted February 27, 2017. From the *Department of Forensic Medicine, Adichunchanagiri institute of

medical sciences, B G Nagara; and Departments of †Forensic Medicine and ‡Pathology, M.S. Ramaiah Medical College, Bengaluru, India.

The authors report no conflict of interest. Reprints: Javaregowda Vinay, MD, Department of Forensic Medicine,

Department of Forensic Medicine, Adichunchanagiri institute of medical sciences, B G Nagara, Karnataka 571448, India.

E-mail: drvinayjgowda@gmail.com.

Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

ISŚŃ: 0195-7910/17/3802–0167

DOI: 10.1097/PAF.000000000000314

may have medicolegal significance. The 3 key manifestations of blunt force injury spectrum are abrasions, lacerations, and contusions. Among which, abrasions are most commonly encountered blunt injuries. Accurate dating of such injury has been an area of considerable research and debate. The body's response to trauma is diverse and is affected by innumerable variables. Multiple factors influence the final appearance of these injuries. They can be arbitrarily viewed as being subject dependent, object dependent, or some combination of the two. Subject-dependent factors are anatomical region, age, and their medical status; object related are type of material/substance involved in causing or surface making contact with the body and time duration of the contact.³

In India, as well as many other developing countries, people usually adopt the "naked eye examination" as a method to date an injury. There is obvious incongruity existing in the standard textbooks, sufficient enough to pose difficulty for dating of injuries on the basis of its appearance to the naked eye. Skin wound healing starts immediately after injury and consists of 3 phases: inflammation, proliferation, and maturation. These phases proceed with a complicated but well-organized interaction between various tissues and cells.⁴ Many cells and biochemical substances are closely involved in the healing process to complete tissue repair after damage. Because of the uncertain and variable results of the gross examination of the wounds, it is important to study the injuries microscopically. Correlating the gross appearance with histological changes of wounds is still indispensable despite the sophisticated methods such as histochemistry and immunohistochemistry for wound dating.⁵ Issues concerned to the wound dating in forensic medicine could be resolved by adopting an overview of wound healing provided that it is simplified and freed from the false impression with rigid standards being set. Hence, this study of wound dating from gross and microscopic examination of abrasion was taken up to look into the accuracy of dating the wounds by comparing with the age determined by color changes and microscopic examination with that of the time of infliction of injury as informed by the police records, hospital records, eyewitness, or relatives and friends.

AIM

This is a study on postmortem wound dating by gross and histopathological examination of abrasions.

OBJECTIVES

To determine the age of abrasions by gross and histopathological techniques.

To correlate gross and histopathological changes in relation to time of injury.

MATERIALS AND METHODS

Source of Data

This study was conducted in the Department of Forensic Medicine, M.S. Ramaiah Medical College, from October 2011 to March 2013, for a period of 18 months.

Am J Forensic Med Pathol • Volume 38, Number 2, June 2017

www.amjforensicmedicine.com | 167

	5		
Age, y	Male, n (%)	Female, n (%)	Total, n (%)
0–10	1 (50.0)	1 (50.0)	2 (100.0)
11-20	6 (100.0)	0 (0.0)	6 (100.0)
21-40	47 (92.2)	4 (7.8)	51 (100.0)
41–60	28 (82.4)	6 (17.6)	34 (100.0)
>60	7 (87.5)	1 (12.5)	8 (100.0)
Total	89 (88.1)	12 (11.9)	101 (100.0)

TABLE 1. Age and Sex Distribution of the Cases

Method of Collection of Data

All fatal cases having well-demarcated wounds and known time of injury were included among the cases subjected to medicolegal autopsy. A total of 101 samples after fulfilling the inclusion criteria were taken up in the study. A standard proforma was used to collect information regarding time of injury, time of death, and associated comorbidities. Consent for tissue section was obtained after detailed interviews with investigating officer, relatives, friends, or whoever witnessed the incident and hospital records of the deceased. Standard autopsy protocol was followed; relevant details of the injuries such as site, size, and color were taken into consideration, and the age of injury was assessed depending on appearance.

Then, injuries were grouped under 7 different time intervals— 0 to 4, 4 to 12, 12 to 24, and 24 to 72 hours; 4 to 6 and 7 to 14 days; and more than 2 weeks—based on the survival period after the infliction of injury. The representative areas of injury with adjacent normal skin were sampled along with control sample from a nearer tissue of injury that was taken and preserved in 10% formalin for a day, then samples were processed, and blocks were created using paraffin. The process of embedding in paraffin blocks allows the sectioning of tissues into very thin $(2-7 \ \mu m)$ sections using a microtome. The slices are thinner than the average cell and are layered on a glass slide for staining by hematoxylin and eosin (often abbreviated as H&E). Then, the slide is viewed under a microscope. Von Gieson staining was performed whenever necessary to confirm the presence of collagen tissue.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria are cases where the time of infliction of injury was known. Exclusion criteria are bodies in the state of decomposition. Ethical clearance was obtained (ref. no. STD-1/EC/0416/2011).

RESULTS AND DISCUSSION

A total of 101 samples were taken up for the study. In the study, of 101 cases, 89 cases (88.1%) were men because they are more into outdoor activities such as driving vehicles, labor work, and so forth, and the remaining 12 cases (11.9%) were women. Most were those in the age group of 21 to 40 years (51 cases), followed by 41 to 60 years (34 cases) as shown in Table 1. The reason was that they form the work group and are more prone to injuries due to road traffic accidents, fall, assaults, and so forth.

A similar observation was made in a study conducted by Sharma et al⁶ on 60 corpses, where the maximum number of cases, that is, 24 cases (40%), belong to the age group of 21 to 30 years, followed by the age groups of 31 to 40, 41 to 50, and 51 to 60 years (13.34% of cases in each group). The minimum number of cases was observed in the age groups of younger than 10, 61 to 70 years, and older than 70 (3.34% of cases in each group). It was observed in another study that, of 35 total cases in the age group of 31 to 40 years, maximum cases belonged to the age group of 31 to 40 years, comprising 25 men and 10 women.⁷

Of the 101 total cases, 89 cases (75.4%) were due to road traffic accident, 10 cases (8.5%) were due to fall from height, 1 case (0.8%) was due to railway injury, and the remaining 1 case (0.8%) was due to assault.

Among the 101 cases, 29 cases (24.6%) were 0 to 4 hours old, 11 cases (9.3%) were of the duration of 4 to 12 hours, 10 cases (8.5%) were 12 to 24 hours old, 18 cases (15.3%) were 24 to 72 hours old, 8 cases (6.8%) were 4 to 6 days old, 16 cases (13.6%) were 7 to 14 days old, and the remaining 9 cases (7.6%) were more than 2 weeks old (Fig. 1).

Gross Changes

One hundred one abrasions were studied (Table 2), among which 33 (32.7%) showed bright red, 22 (21.8%) showed reddish scab, 16 (15.8%) showed brownish scab, 3 (3.0%) showed dark brown scab, 10 (9.9%) showed black scab, and in 13 injuries (12.9%), the scab had fallen off at margins. In 4 injuries (4.0%), the scab had fallen off completely.

Bright red color was observed in 33 abrasions; 29 (87.9%) of these injuries were 0 to 4 hours old. The remaining 4 injuries were 4 to 12 hours old, more so between 4 and 5 hours, and were located in the less vascular areas of the extremities. No comorbidities were associated in any of these cases. Bright redness was observed earliest at 10 minutes and latest up to 5 hours. This was in agreement with the study conducted by Sharma et al where they found redness from 10 minutes up to 7 hours. In contrary, it was observed in another study that most cases on the first day were dark red instead of bright red in color.⁷



FIGURE 1. Distribution based on survival period of cases.

168 www.amjforensicmedicine.com

TABLE 2.	Distribution	of Abrasions I	oy Its Gro	oss Changes	According t	o Duration of	Time
			,				

	Gross Changes									
Age of Injury	Bright Red, n (%)	Reddish Scab, n (%)	Brownish Scab, n (%)	Dark Brown Scab, n (%)	Black Scab, n (%)	Scab Margin Falling Off, n (%)	Scab Fallen Off Completely, n (%)	Total, n (%)		
0–4 h	29 (87.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	29 (28.7)		
4–12 h	4 (12.1)	7 (31.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (10.9)		
12–24 h	0 (0.0)	8 (36.4)	2 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (9.9)		
24–72 h	0 (0.0)	7 (31.8)	10 (62.5)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	18 (17.8)		
4–6 d	0 (0.0)	0 (0.0)	4 (25.0)	2 (66.7)	1 (10.0)	1 (7.7)	0 (0.0)	8 (7.9)		
7–14 d	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (70.0)	8 (61.5)	1 (25.0)	16 (15.8)		
>2 wk	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	4 (30.8)	3 (75.0)	9 (8.9)		
Total	33 (100.0)	22 (100.0)	16 (100.0)	3 (100.0)	10 (100.0)	13 (100.0)	4 (100.0)	101 (100.0)		
In this table,	gross changes	in the abrasions	with the age of	injury were asse	essed, and it was	s found to be statistical	ly significant ($P < 0.00$	1). $\chi^2 = 2.32$,		

In this table, gross changes in the abrasions with the age of injury were assessed, and it was found to be statistically significant (P < 0.001). $\chi^2 = 2.32$, df = 36, P < 0.001.

Reddish scab was noted in 22 injuries, of which 8 injuries (36.4%) were 12 to 24 hours old. The remaining 14 were distributed equally (31.8% each) in the durations of 4 to 12 and 24 to 72 hours. Reddish scab was observed earliest at 6 hours and latest up to 68 hours. A similar observation was made by Sharma et al⁵ in their study. An author also quotes that reddish scab forms by 8 to 24 hours.⁸

Brownish scab was noted in 16 injuries, of which 10 injuries (62.5%) were 24 to 72 hours old; the remaining 2 were 12 to 24 hours old, and 4 were 4 to 6 days old. The earliest brownish scab was noted at 18 hours in 2 injuries, where both injuries were small in size and the deceased was young who did not have comorbidities. The latest appearance of the brownish scab was at 132 hours (5.5 days). A similar observation was made by Sharma et al,⁵ and an author also quotes that the scab looks brownish by the second and third days.⁸ This was in contrast with the study conducted by Kumar et al⁷ wherein they observed that, on the third day, most injuries were dark red in color instead of brownish.

The dark brown scab was noted in 3 injuries, of which 2 injuries (66.7%) were 4 to 6 days old and the remaining was 24 to 72 hours old. The earliest dark brown scab was noted at 44 hours, where the injury was situated over the face (more vascular area). The other 2 injuries were at 97 and 144 hours; however, their sizes $(2 \times 1 \text{ and } 5 \times 3 \text{ cm})$ were small, and the deceased did not have any comorbidity. In a similar study, Sharma et al found a hard

TABLE 3. Distribution of Abrasions by Its Microscopic Changes

brown scab 27 hours onward, and an author also quotes that, by the fourth and fifth days, the scab looks dark brown.⁸

However, this was in disagreement with another study where they observed a dark red scab instead of a dark brownish scab on the fifth day.⁷

The black scab was noted in 10 injuries, of which 7 injuries (70.0%) were 7 to 14 days old, 1 injury was 4 to 6 days old, and 2 were observed at more than 2 weeks. The black scab was seen only after 5 days, was seen at 21 days in a deceased who had no comorbidities, and was situated in the leg (less vascular area). In another case, a black scab was noted at 35 days in an elderly man, who was poorly nourished and comatose, died of secondary infections consequent to sustained head injury, and had a history of diabetes and hypertension.

A scab fallen at the margin was seen in 13 injuries, of which 8 injuries (61.5%) were of 7 to 14 days and the remaining 4 were more than 2 weeks old. The fall of scab was noted only after 6 days and was seen up to 15 days in a healthy individual where injury was located in the lower extremity (knee). In the remaining 3 cases, there was significant delay of fall of scab observed up to 45 days. All 3 of them were experiencing septicemia. In 4 injuries, the scabs had fallen off completely, of which 3 injuries (75.0%) were more than 2 weeks old, and in the remaining injury, the scab had fallen off at 11 days itself. In a healthy individual, it took up to 17 days for the scab to fall off completely. However, in a comatose

Microscopic Scoring												
Age of Injury	0, n (%)	1, n (%)	2, n (%)	3, n (%)	4, n (%)	5, n (%)	6, n (%)	7, n (%)	8, n (%)	9, n (%)	10, n (%)	Total, n (%)
0–4 h	23 (76.7)	1 (100)	5 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	29 (28.7)
4–12 h	6 (20.0)	0 (0.0)	3 (37.5)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (10.9)
12–24 h	1 (3.3)	0 (0.0)	0 (0.0)	1 (33.3)	8 (53.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (9.9)
24–72 h	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (46.7)	6 (85.7)	1 (50.0)	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)	18 (17.8)
4–6 d	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	5 (38.5)	2 (20.0)	0 (0.0)	0 (0.0)	8 (7.9)
7–14 d	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	3 (23.1)	7 (70.0)	4 (40.0)	1 (50.0)	16 (15.8)
>2 wk	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	1 (10.0)	6 (60.0)	1 (50.0)	9 (8.9)
Total, %	30 (100)	1 (100)	8 (100)	3 (100)	15 (100)	7 (100)	2 (100)	13 (100)	10 (100)	10 (100)	2 (100)	101 (100)

Cross-tabulation between age of injury and microscopic changes in abrasion was found to be statistically significant (P < 0.001). $\chi^2 = 2.45$, df = 60, P < 0.001.

© 2017 Wolters Kluwer Health, Inc. All rights reserved.

	Microscopic Scoring											
Gross Changes	0, n (%)	1, n (%)	2, n (%)	3, n (%)	4, n (%)	5, n (%)	6, n (%)	7, n (%)	8, n (%)	9, n (%)	10, n (%)	Total, n (%
Bright red	24 (72.7)	1 (3.0)	8 (24.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	33 (100.0)
Reddish scab	6 (27.3)	0 (0.0)	0 (0.0)	3 (13.6)	9 (40.9)	2 (9.1)	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	22 (100.0)
Brownish scab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (37.5)	3 (18.8)	2 (12.5)	4 (25.0)	1 (6.2)	0 (0.0)	0 (0.0)	16 (100.0)
Dark brown scab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)
Black scab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	2 (20.0)	4 (40.0)	2 (20.0)	1 (10.0)	10 (100.0)
Margins starts falling off	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)	5 (38.5)	5 (38.5)	1 (7.7)	13 (100.0)
Scab fallen off completely	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	4 (100.0)
Total	30 (29.7)	1 (1.0)	8 (7.9)	3 (3.0)	15 (14.9)	7 (6.9)	2 (2.0)	13 (12.9)	10 (9.9)	10 (9.9)	2 (2.0)	101 (100.0)

TABLE 4. Correlation of Gross Changes With Microscopic Changes of Abrasions

patient, who had comorbidities such as diabetes and septicemia, the fall of scab (complete) was observed on the 27th day. In a similar study by Sharma et al, they found that the black scab started separating 7 days onward,⁹ and the authors quote that, by the sixth day, it turned blackish and started falling off from the margins. A big scab takes a few more days to fall off.⁸ However, this was in disagreement with the study where they observed a dark brown scab instead of a black scab on the ninth day.⁷

The Scoring for Microscopic Changes as Given in the Following Order

- 0. Hemorrhage and/or congestion of vessels
- 1. Edema formation
- 2. Margination of polymorph cells
- 3. Early infiltration of polymorph cells
- Predominant polymorph infiltration with poorly differentiated mononuclear cells
- 5. Predominant mononuclear cell infiltration
- 6. Appearance of fibroblast
- 7. Granulation tissue with rich leukocyte infiltration and much fibroblasts
- 8. Collagen tissue deposition

TABLE 5.	Earliest,	Routine,	and	Latest	Appearances	of	Gross
Changes of	of Abrasic	ons					

Gross Changes	Earliest Appearance	Routine Appearance	Latest Appearance
Bright red	10 min	0–4 h	5 h
Reddish scab	6 h	12–24 h	68 h
Brownish scab	18 h	24–72 h	132 h
Dark brown scab	44 h	4–6 d	144 h
Black scab	120 h (5 d)	7–14 d	21 d*
Scab fallen off at margin	144 h (6 d)	7–14 d	15 d†
Scab fallen off completely	11 d	>2 wk	17 d‡

*With comorbid conditions, a black scab was observed up to 35 days. †With comorbid conditions, a scab has fallen off at margin up to 45 days.

 $\ddagger With comorbid conditions, a scab has fallen off completely up to 27 days.$

9. Cellular reaction subsides; fibroblast are more active with increased collagen formation (regression phase of injury)

10. Organized pustule formation in the presence of infection

Microscopic Changes

Table 3 shows among 30 abrasions, which showed only hemorrhage and/or congestion of vessels, 23 (76.7%) were 0 to 4 hours old, 6 were 4 to 12 hours old, and one was 12 to 24 hours old. The presence of congestion of vessels and/or hemorrhage was observed from 10 minutes and lasts up to 6 hours, except in 2 where it was observed at 11 and 14 hours. The earliest formation of edema was noted at 15 minutes. Margination of polymorphs was observed during 0 to 4 hours in 5 (62.5%) of 8 injuries. This was seen earliest at 30 minutes and lasted up to 5 hours. Early polymorphs infiltration was observed in 3 injuries, of which 2 injuries (66.7%) were 4 to 12 hours old. It was seen earliest at 6 hours. A similar observation was made in a study by Sharma et al⁵ where early polymorph infiltration was found at 7 hours, and another author also quotes that the margination of poly-morph occurs by 4 hours.¹ An author quotes that the polymorph infiltration in a perivascular fashion was seen from 2 to 6 hours.¹⁰ On the contrary, in another study, the author comments that the earliest appearance of polymorphs was observed at 20 to 30 minutes after an injury.¹¹

TABLE 6. Earliest and Routine Appearances of Common

 Histologically Detected Changes of Abrasions

Microscopic Changes	Earliest Appearance	Routine Appearance
Congestion/hemorrhage	10 min	0–4 h
Edema formation	15 min	0–4 h
Margination of polymorph cells	30 min	0–4 h
Early polymorph infiltration	6 h	4–12 h
Predominant polymorph infiltration	12 h	12–24 h
Mononuclear cell infiltration	24 h	24–72 h
Appearance of fibroblast	71 h	71–78 h
Granulation tissue deposition	72 h	4–6 d
Collagen formation	96 h (4 d)	7–14 d
Regression phase	213 h (9 d)	>2 wk



FIGURE 2. Photograph of the skin from control site showing layers of the skin (H&E).

Prominent polymorph infiltration was observed in 15 injuries, of which 8 (53.3%) were 12 to 24 hours old and the remaining 7 (46.7%) were 24 to 72 hours old. It was noted earliest at 12 hours, and progressive increase was noted in the 24- to 72-hour-old injuries. A similar observation was made by Ross and Benditt,¹² where, in less than 12 hours, the wound was rich in polymorph nuclear leukocytes, and the numbers of those cells increased for the first 24 hours to a maximum. An author also comments that infiltration progressively increases from 12 to 18 hours.¹⁰ A similar observation was made by Sharma et al⁵ in their study.

Mononuclear cell infiltration was first noted at 24 hours, and increased infiltration was observed in 6 injuries during 24 to 72 hours. It was noted that predominant mononuclear infiltration was between 35 and 50 hours in 5 injuries. The earliest regenerative change of epithelium was noted at 2 days. The active fibroblasts were evident only after 71 hours. In a similar study by Sharma et al,⁵ early mononuclear infiltration was observed at 13 hours itself, whereas we noticed that in our study at 24 hours. Similar observations were made by an author where the earliest mononuclear infiltration was noted at 20 hours.¹³ Another author quotes that the regeneration of epithelium starts as early as 30 hours and is clearly visible by 72 hours in most cases.¹⁰

The granulation tissue deposition was noted in 13 injuries, of which 5 (38.5%) were 4 to 6 days old, 4 were 24 to 72 hours old, and the remaining 3 injuries were 7 to 14 days old. The early granulation tissue (neovascularization) was noted at 72 hours, and an increase in the deposition of granulation tissue was observed routinely by 4 to 6 days. This observation is in agreement with an author who found that the granulation tissue formation is seen by 5 to 8 days,¹⁰ and another author also quotes the similar finding.¹

The collagen tissue was noted in 10 injuries, of which 7 injuries (70.0%) were 7 to 14 days old. The earliest collagen formation was seen at 96 hours and more commonly in 7- to 14-day-old injuries. This was in agreement with a study where collagen formation started after the third day. A similar observation was made in another study by Ross and Benditt,¹² and an author quotes that, at 3 to 6 days, collagen formation begins and later increases in density.¹ The regression phase was noted in 10 injuries, of which 6 (60.0%) were more than 2 weeks old and the remaining injuries were 7 to 14 days old. The earliest regression phase was noted at 9 days and was more common in injuries of more than 2 weeks old. In a similar study by Ross and Benditt, at 14 days, the morphologic features were parallel to those of the earlier periods.¹³ However, another author quotes that the regression phase will start by the 12th day.¹⁰ In 2 injuries, the formation of organized pustule rich in polymorphs was noted during the period of 7 to 14 days and more than 2 weeks old.

Correlation of Gross Changes With Microscopic Changes

Table 4 shows among 33 abrasions of bright red color, 24 (72.7%) had hemorrhage and/or congestion of vessels, 1 was associated with edema, and the remaining showed margination of polymorphs.

A reddish scab was noted in 22 abrasions. Among them, 9 (40.9%) had predominant polymorphs infiltration, 6 had only hemorrhage and/or congestion of vessels, 3 had margination of polymorph cells, 3 had significant mononuclear cell infiltration, and 2 showed granulation tissue deposition.

A brownish scab was noted in 16 abrasions, among which 6 (37.5%) had predominant polymorph cell infiltration, 4 had granulation tissue, 3 had mononuclear cell infiltration, and 2 injuries had active fibroblasts.

A dark brown scab was noted in 3 abrasions, among which 2 (66.7%) showed dense granulation tissue.

A black scab was noted in 10 abrasions; among them, 4 injuries (40%) had collagen tissue, 2 injuries showed granulation tissue, 2 injuries were in the regression phase, and in 1 case, there was organized pustule.

A scab had fallen off at margin in 13 abrasions; most of them showed collagen tissue deposition or were in the regression phase (38.5% each), and in 1 injury, there was organized pustule.

Scabs had fallen off completely in 4 injuries, where most of these injuries were in the regression phase (75%).

By comparing gross changes with microscopic changes, among the injuries of 4 to 6 days in duration, 2 (25%) of 8



FIGURE 3. A, Abrasion with bright red color (2 hours old). B, Photograph of the injury showing loss of epidermis and congested vessels without any inflammatory response (H&E).



FIGURE 4. A, Abrasion covered with a brownish scab (65 hours old). B, Photograph of the injury showing early regenerative changes of the epithelium—dermal edema with dense inflammatory response (H&E).

abrasions showed a dark brown scab, whereas by histological study, 5 (62.5%) of them showed granulation tissue, which confirmed the age of injury to be more than 4 days.

Among the injuries of more than 2 weeks old, 3 (33.3%) of 9 abrasions showed a scab that has fallen off completely, whereas by histological study, 66.7% of the injuries showed dense collagen tissue with decreased cellular reaction, which confirms the injury as more than 2 weeks old.

CONCLUSIONS

A postmortem study of wound dating by gross and histopathological examination of abrasions was carried out in the Department of Forensic Medicine, M.S. Ramaiah Medical College, from October 2011 to March 2013. A total of 101 samples (injuries) were taken up for the study. Most of them were in the age group of 21 to 40 years (50.5%), and men constituted more than three fourths (88.1%) of the cases. Furthermore, 75.4% of the cases were due to road traffic injuries, and the remaining were due to railway injuries, fall from height, and so forth.

The results concluded are as follows: on gross examination, bright red color was seen in injuries less than 5 hours old. The reddish scab was seen up to 68 hours. A brown scab was seen after 18 hours. A dark brown scab was observed only after 44 hours. A black scab was observed in the injuries more than 5 days old. It took at least 6 days for the scab to fall from the margin and 11 days to fall off completely (Table 5).

On microscopic examination, hemorrhage and congestion of vessels were seen in injuries less than 14 hours old. Predominant polymorph cell infiltration was observed only after 6 hours. Mononuclear cell infiltration was observed in injuries more than 1 day old. The granulation tissue was seen after 72 hours. Collagen tissue formation was observed after 4 days (Table 6).

Among 4- to 6-day-old abrasions, the accuracy of wound dating was increased from 25% to 62.5%; similarly, among those more than 2 weeks old, the accuracy was increased from 33.3% to 66.7% by subjecting it for histological study.

Role of Comorbid Conditions on Wound Healing

- In comatose patients with or without other comorbidities such as diabetes, hypertension, sepsis, malnutrition, and so forth, delay in the scab formation was observed.
- By microscopy, the infiltration of inflammatory cells (polymorphs and mononuclear cells) was observed in the infected wounds even up to 45 days. Hence, these factors are to be considered while giving opinion with regard to dating of the injury.
- To conclude, although gross features of the injuries provide a rough estimate regarding their age, histological examination will confirm or gives a more accurate timing of their occurrence, thus aiding in the administration of justice.

LIMITATIONS

- In individuals with dark brown complexion, the early color changes are difficult to appreciate clearly, and more so, this type of naked eye observation is subjective.
- The degree of possible observer variation was not assessed.

SCOPE FOR FURTHER STUDY

• Further studies on enzyme histochemistry, biochemical assays, and fluorescence studies can be conducted and



FIGURE 5. A, Abrasion covered with a black scab (5.5 days old). B, Photograph of the injury showing regenerative changes in the epithelium with subepithelial granulation tissue deposition (H&E).



FIGURE 6. Photograph of the injury showing regenerative changes in the epithelium—dense active fibroblasts with collagen tissue deposition (H&E) (6 days old).



FIGURE 7. Photograph of the injury showing pustule rich in inflammatory cells (H&E), on the 27th day.

correlated with microscopic changes. This will help in removing subjectivity and bring more objectivity in the observation and interpretation.

RECOMMENDATIONS

To the Autopsy Surgeon

In an Indian scenario for dating of injuries, we rely on naked eye examination (subjective) of gross changes, which gives a rough estimate regarding the age; thus, subjecting the samples for histological examination would be more accurate.

 In cases with multiple injuries over different sites, the healing process may vary because of various factors, and thus, opining the age of the injury would be difficult and would not correlate with the time of infliction of the injury as alleged by the police or relatives; hence, the autopsy surgeon should exercise caution while opining the age.

• In histopathology slides during examination, the presence of artifacts in the form of sand, mud particles, glass pieces, and so forth is to be kept in mind.

ACKNOWLEDGMENT

The authors thank the Department of Forensic Medicine and Pathology, M.S. Ramaiah Medical College, Bengaluru.

REFERENCES

- Knight B, Saukko P, (eds). The pathology of wounds. In: *Knight's Forensic Pathology*. 3rd ed. London, England: Arnold Publishers; 2004: 166–169.
- Pilling ML, Vanezis P, Perrett D, et al. Visual assessment of the timing of bruising by forensic experts. *J Forensic Leg Med.* 2010;17(3): 143–149.
- Dolinak D, Matshes WE, Lew OE, (eds). Blunt force injury. In: *Forensic Pathology Principles and Practice*. London, England: Elsevier Academic Press; 2005:121.
- Kondo T, Ishida Y. Molecular pathology of wound healing. *Forensic Sci Int.* 2010;203:93–98.
- Sharma A, Dikshit PC, Aggrawal A, et al. A post mortem study of histopathological findings to determine the age of abrasion and laceration. *J Forensic Med Toxicol.* 2010;27(1):43–46.
- Sharma A, Khanna SK, Aggrawal A, et al. A histopathological study to determine the age of contusion. *J Punjab Acad Forensic Med Toxicol*. 2010; 10:17–19.
- Kumar L, Chaitanya BV, Agarwal SS, et al. Age related changes in mechanical and thermal injuries—a post mortem study. *J Indian Acad Forensic Med.* 2011;33(2):149–151.
- Nandy A. Mechanical injuries. In: *Principles of Forensic Medicine and Toxicology*. 3rd ed. New Delhi, India: New Central Book Agency; 2000:352.
- Cummings PM, Trelka DP, Springer KM. Atlas of Forensic Histopathology. Cambridge, England: Cambridge University Press; 2011:1–10.
- Dimaio VJ, Dimaio D, Geberth VE, (eds). Blunt trauma wounds. In: *Forensic Pathology*. 2nd ed. Boca Raton, FL: CRC Press; 2001:94–98.
- 11. Betz P. Histological and enzyme histochemical parameters for the age estimation of human skin wounds. *Int J Leg Med.* 1994;107:60–68.
- Ross R, Benditt EP. Wound healing and collagen formation: I. Sequential changes in components of guinea pig skin wounds observed in the electron microscope. J Biophys Biochem Cytol. 1961;11(3):677–700.
- Payne JJ, Busttil A, Smack W, (eds). Pathophysiology of wound healing. In: *Forensic Medicine, Clinical and Pathological Aspects*. London, England: Greenwich Medical Media Ltd; 1977:83–87.