ORIGINAL ARTICLE

Indian Journal of Forensic Medicine and Pathology
Volume 16, Number 3, July-September 2023
DOI: https://dx.doi.org/10.21088/ijfmp.0974.3383.16323.2

Estimation of Age of Abrasion by Histopathology Examination: A Cross-Sectional Study

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How to cite this article:

Shikha Shrivastava, Prateesh Shukla, Yashwant Kumar Singh, *et al.*/Estimation of Age of Abrasion by Histopathology Examination: A Cross-Sectional Study. Indian J Forensic Med Pathol.2023;16(3):179-184.

ABSTRACT

Context: In India, abrasions are usually dated by the naked-eye observation of gross changes. Various researches consider the dating of abrasion by naked-eye examination as an uncertain and variable method. This may have significant medical and legal ramifications. To improve the accuracy of the dating of abrasion, histopathology profiling has been suggested.

Aims: To estimate the age of abrasion by histopathology examination.

Settings and Design: This cross-sectional study was conducted from 1st January 2021 to 30th June 2022.

Methods and Materials: Total 102 abrasions from subjects aged 4 to 80 years were correlated with the time frame of the occurrence of different microscopic changes that follow the abrasion. Abrasions ranging from 0.02 hours to >336 hours were studied, and based on the duration of infliction, abrasions were divided into 10 groups. The representative area of abrasion with adjacent normal skin with an underlying soft tissue of thickness 0.5 cm to 1 cm was dissected and a control sample was taken of intact skin and preserved in 10% formalin solution for fixation. The specimen was processed, and a histopathology slide was prepared and examined. Routine haematoxylin & eosin (H&E) staining done. Van Gieson's stain was used to confirm the presence of collagen.

Statistical analysis used: Data were analysed using SPSS version 20.0 software. $P<0.05$ was considered significant. Descriptive data were expressed as frequency; for the age group of abrasions (quantitative data), mean and SD were calculated; Pearson's chi-square test of independence was used to compare the microscopic changes in abrasion with the age of infliction.

Results: A significant relationship was observed between the age of abrasion and histopathology findings ($X^2_{81}=552.92$, $P<0.001$).

Conclusions: The reliability and accuracy of dating of abrasion increases if a histopathology examination is performed along with gross examination. To opine the age of injury accurately, the autopsy surgeon should subject the samples to histopathology examination.

Keywords: Abrasion; Age of abrasions; Autopsy; Histopathology.

Key Messages: To opine the age of abrasion accurately, the autopsy surgeon should subject the samples to histopathology examination.

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Received on: *06-04-2023*

Accepted on: *19-09-2023*

INTRODUCTION

The determination of the age of an injury is one of the most important aspects of medico-legal examination. Significant medical and legal ramifications could result from their interpretation, such as, the inclusion or exclusion of a suspect as a criminal offender.[1] In forensic autopsy practise, injuries due to blunt impact are frequent, and proper interpretation of wounds is crucial for reliable medical testimony.[2] Among blunt traumas, abrasion is the most common,[3] and it is the most important tool for determining the age of the injury.

The dating of injuries has been a topic of disagreement among researchers. In India, injuries are usually dated by naked eye examination.[4] Colour changes in abrasion are frequently used for estimating the age of injury; however, some authors consider it as an uncertain and variable method for dating the wound due to observer bias. To improve the accuracy of the dating of abrasion, histopathology profiling has been suggested.[4,5]

Hence, this research was conducted to date an abrasion by histopathology examination.

MATERIALS AND METHODS

This cross-sectional study was conducted between 1st January 2021 and 30th June 2022 after obtaining ethical clearance from the Institutional Ethics Committee (IEC).

The study population was dead bodies brought by the police for a medico-legal autopsy. Dead bodies having a well demarcated abrasion and a known time of infliction were included. Dead bodies with infected abrasions and dead bodies in the state of decomposition were excluded from the study.

According to the selection criteria, 102 cases, between 1 and 80 years were selected for the study. Written informed consent to collect skin samples of representative areas of abrasions, with adjacent normal skin, along with control samples from nearer tissue, and their histopathology examination, was obtained from relatives accompanying the deceased. Information regarding the time of injury and death, hospitalisation, and associated comorbidities were noted. Based on the age of infliction, abrasions were grouped under 10 different time intervals i.e. ≤12 hours, 13 to 24 hours, 25 to 48 hours, (2nd day), 49 to 72 hours (3rd day), 73 to 96 hours (4th day) 97 to 120 hours (5th day), 121 to 144 hours (6th day), 145 to 168 hours (7th day) 169 to 336 hoyrs (8-14 days), and > 336 hours (>2 weeks).

The representative area of abrasion with adjacent normal skin with an underlying soft tissue of thickness 0.5 cm to 1 cm was dissected and a control sample was taken of intact skin and preserved in 10% formalin solution for fixation. The specimen was processed, slide prepared, and examined. Routine haematoxylin & eosin (H&E) staining done. Van Gieson's stain was used to confirm the presence of collagen. After staining, the slides were viewed under an Olympus microscope with Magvision software, and the findings were observed and recorded for both the sample and control.

Data were analysed using SPSS version 20.0 software. $P<0.05$ was considered significant. Descriptive data were expressed as frequency. For the age group of abrasions (quantitative data), mean and SD were calculated. Pearson's chi-square test of independence was used to compare the microscopic changes in abrasion with the age of infliction.

RESULTS

In this cross-sectional study, the sample size was 102 with age range of the study subjects was from 4 years to 80 years. Among 102 cases, 84 (82.35%) were male and 18 (17.65%) were female. The abrasions were most commonly observed in the age group 31 to 40 years (25/102, 24.51%) followed by 21 to 30 years (24/102, 23.53%).

In our study, edema formation was the most common (37/102, 36.27%) and margination of polymorph cells was the least common (1/102, 0.98%) microscopic findings. In our study, postinfliction congestion of vessels and haemorrhage was the first observed microscopic change (range 0.5–5 hours). Edema formation was observed earliest from 0.02 hours to the latest up to 35.75 hours. After that, polymorphs dominate the microscopic profile with margination (5.3 hours), early polymorph infiltration (range 1.67–32 hours), and predominant polymorph infiltration (range 12.05–29.08 hours). Subsequently, mononuclear cell infiltration (range 27.08–69.05 hours) and appearance of fibroblasts (range 72.17–92.17 hours) was observed. Granulation tissue deposition was seen from 100.42 to 165.67 hours postinfliction. Collagen was observed microscopically from 157 to 333.33 hours. The last phase of healing was the regression phase, which was seen in abrasion >336 hours old.

In our study, half of the cases (51/102, 50%) had an abrasion of age ≤12 hours (mean 3.26, SD 2.91). In abrasions ≤12 hours, edema was the most common microscopic finding (36/51, 70.59%), and

margination and early infiltration of polymorphs were also seen. Between 13 and 24 hours (mean 16.82, SD 3.22) after injury, all the abrasions showed predominant infiltration by polymorphs. On the second day (mean 32.75, SD 4.67 hours) mononuclear cells were seen in the majority of cases (5/9, 55.56%). Edema formation and polymorph cells were seen microscopically in the remaining cases (4/9, 44.44%). All the abrasions on the third day (mean 63.33, SD 5.39 hours) and the fourth day (mean 82.39, SD 8.54 hours) postinfliction showed mononuclear infiltration and appearance of fibroblasts, respectively. Granulation tissue deposition was observed in all abrasions on the fifth and sixth days, and the majority (4/5, 80%) of abrasions on the seventh day. Collagen formation was observed microscopically in all abrasions from 8th to 14th days (mean 240.33, SD 70.98 hours). Regression in abrasion was seen only after 14 days (336 hours).

In this study, a statistically significant relationship between the age of abrasion and histopathology findings was found (X^2_{81}=552.92, P<0.001).

Table 1: Distribution of cases by Histopathology Findings

Histopathology Findings	No. of cases, N (N%)	Age of Abrasion, Mean (SD) (hours)
Congestion/Haemorrhage	5 (4.9%)	1.79 (1.86)
Edema formation	37 (36.27%)	3.43 (5.93)
Margination of Polymorph cells	1 (0.98%)	5.3 (0)
Early Polymorph infiltration	11 (10.78%)	10.91 (9.76)
Predominant Polymorph infiltration	7 (6.86%)	18.57 (5.49)
Mononuclear cell infiltration	8 (7.84%)	45.05 (15.94)
Appearance of Fibroblast	8 (7.84%)	82.39 (8.54)
Granulation tissue deposition	12 (11.76%)	127.65 (23.84)
Collagen formation	8 (7.84%)	229.92 (72.02)
Regression phase	5 (4.9%)	664.88 (345.89)
Total	102 (100%)	79.48 (166.06)

Table 2: Distribution of cases by Age of Abrasion and Histopathology Findings

Age of Abrasion	No. of cases (n)	Age of Abrasion Mean (SD)	Histopathology Findings	No. of cases (n)
≤12 h	51	3.26 (2.91) h	Congestion/Haemorrhage	5
			Edema formation	36
			Margination of Polymorph cells	1
			Early Polymorph infiltration	9
13 to 24 h	6	16.82 (3.22) h	Predominant Polymorph infiltration	6
25 to 48 h (2nd day)	9	32.75 (4.67) h	Edema formation	1
			Early Polymorph infiltration	2
			Predominant Polymorph infiltration	1
			Mononuclear cell infiltration	5
49 to 72 h (3rd day)	3	63.33 (5.39) h	Mononuclear cell infiltration	3
73 to 96 h (4th day)	8	82.39 (8.54) h	Appearance of Fibroblast	8
97 to 120 h (5th day)	5	105.57 (6) h	Granulation tissue deposition	5
121 to 144 h (6th day)	3	126.85 (7.78) h	Granulation tissue deposition	3

table cont....

145 to 168 h (7th day)	5	156.09 (9.55) h	Granulation tissue deposition	4
			Collagen formation	1
169 to 336 h (8th to 14th day)	7	240.33 (70.98) h	Collagen formation	7
>336 h (>2 wks)	5	664.88 (345.89) h	Regression phase	5
Total	102	79.48 (166.06) h		102

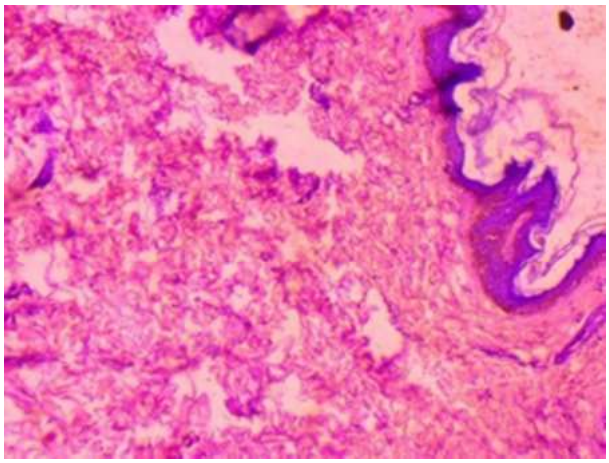

Fig. 1: Edema, congestion, and haemorrhage, H&E (10x).

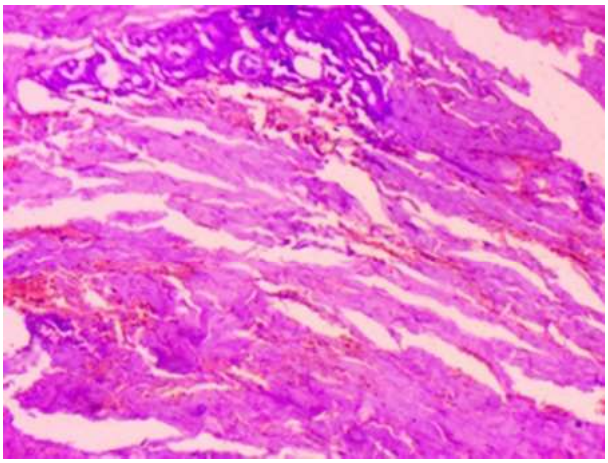

Fig. 2: Polymorph infiltration, H&E (10x).

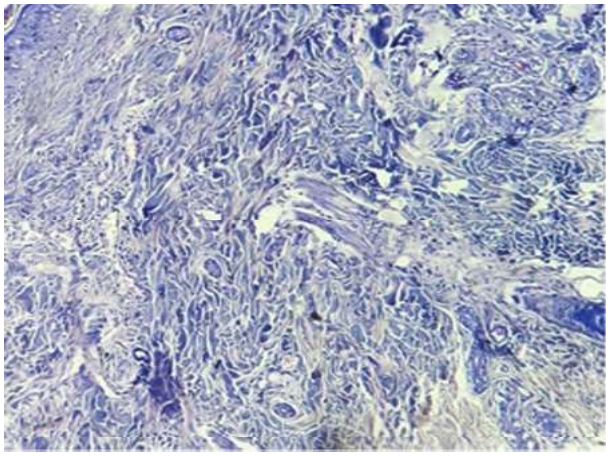

Fig. 3: Mononuclear cells, H&E (10x).

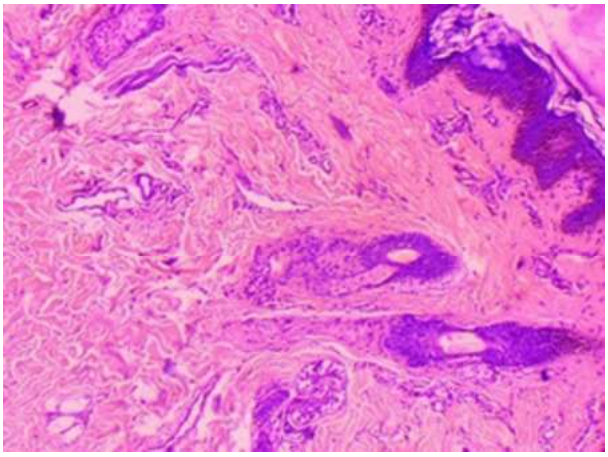

Fig. 4: Fibroblast cells, H&E (10x).

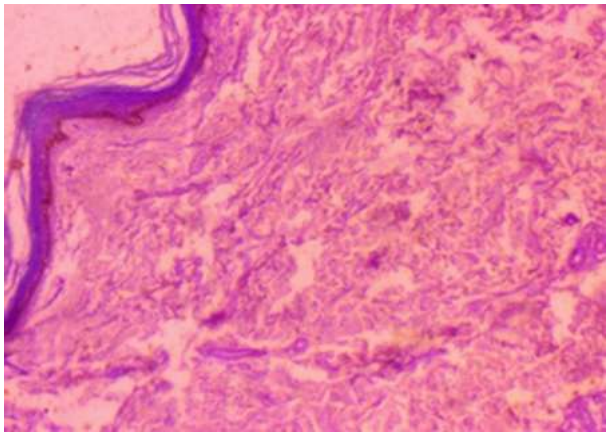

Fig. 5: Granulation tissue, H&E (10x).

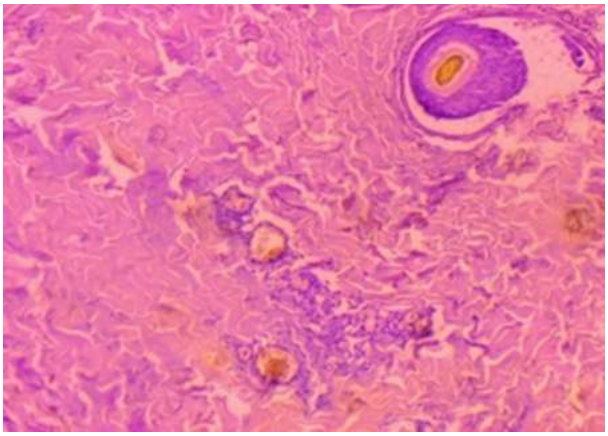

Fig. 6: Collagen, Van Gieson (10x).

DISCUSSION

Sample size in our study (N=102) was higher compared to other studies.[4–10] We observed a male preponderance (M:F: 4.67:1) similar to other researchers.[4,5,7–11] The observed male preponderance can mainly be attributed to the fact that men are more prone to injuries since they are more involved in outdoor activities such as driving vehicles, labour workers, etc.

In our study, the age range of the study subjects was from 4 years to 80 years. The age range of subjects in other research was from 1 year to more than 60 years.[4,7,9–11] A few research did not include extreme age groups and included subjects only between the ages of 18 and 60 years.[5,8]

We observed congestion of vessels and haemorrhage in abrasions from 0.5 to 5 hours after wounding. Research reported haemorrhage and/or congestion of vessels in the majority abrasion that were 0-4 hours old, supporting our findings.[4,5,10]

Edema formation, in our study, was seen microscopically at mean 3.43 (SD 5.93; range 0.02–35.75) hours. In a few studies, edema was observed in 0 hour to 4 hours old abrasion.[4,5] Although the observations in these research were similar to our study, the sample size in this group was too small to compare with our study or to draw any meaningful conclusion.

In this study, margination of polymorph cells was observed only in one abrasion aged 5.3 hours, such a small sample size in this group prevented us from drawing any meaningful inference. However, research and textbooks reported an earlier margination of polymorph cells in the majority of abrasions that were 0 to 4 hours old.[4,5,10,12]

We found early polymorph infiltration from 1.67 to 32 hours (mean 10.91, SD 9.76). Our observations were in agreement with the research[4,5,10] that found early polymorph infiltration in the majority of cases in 4 to 12 hours old abrasions. Relatively earlier appearance of polymorph infiltration in a perivascular fashion, from 2 to 6 hours, was quoted by one author,[13] which is in the purview of our finding. However, the earliest appearance of neutrophils, at about 20 to 30 minutes after wounding, was reported in one research.[14]

We observed predominant polymorph infiltration between 12.5 and 29.08 hours. Our observation was in agreement with the studies[4,5,10,15] that found predominant polymorph infiltration in the majority of cases between 12 hours and 24 hours old abrasions. Contrary to the above research, Sharma *et al.*[6] observed the earliest polymorph infiltration at 7 hours and the latest up to 3 days. A progressive increase in polymorphs was observed from 12 hours to 18 hours[13] and from 24 to 72 hours[4,10] in a few studies. In our research, we did not study the progressive increase in polymorph infiltration.

Mononuclear cell infiltration, in the majority of cases, was reported in studies, after 24 hours up to 72 hours of wounding.[4,5,10] These researches are in agreement with our findings (range 27.08-69.05 hours). Compared to our observation, a few researchers reported an earlier infiltration of mononuclear cells at 13 hours[6] and at 20 hours[16] respectively.

We found fibroblasts to appear between 72.17 and 92.17 hours. A few research reported the appearance of fibroblasts in the majority of cases between 24 hours and 6 days, which is a wider range compared to ours. However, in these studies, the sample size in this group was small to draw meaningful conclusions.[4,5,10] Another research also reported an earlier fibroblastic proliferation, from 27 hours onwards.[6]

We observed granulation tissue formation between 100.42 and 165.67 hours. Similar to our study, research has found that the majority of 4 to 6 days old abrasions show granulation tissue formation.[4,5,10] In contrast to our study, Siddiqui *et al.*[5] observed granulation tissue from 24 hours up to more than 2 weeks. A few authors have quoted granulation tissue formation in 5-8 days.[12,13] Betz P[16] quoted an earlier appearance at about 3 days after wound infliction.

We found collagen tissue in abrasion between 157 and 333.33 hours. Similar to our study, other researchers have observed collagen formation in the majority of the abrasions that were 7-14 days old.[4,5,10] In contrast, Sharma *et al.*[6] observed collagen formation that starts after the third day and persists up to 25 days. Siddiqui *et al.*[5] observed collagen formation from 4 days to more than 2 weeks. Saukko P[12] and Ross *et al.*[15] also quoted that, at 3 to 6 days collagen formation begins and later increases in density.

Our observations were supported by Ross *et al.*,[15] who observed the regression phase at 14 days. A similar observation was noted in other research[4,5,10] in which the majority of abrasions showed regression in more than 2 weeks postinfliction. Other research[4,10] observed that the earliest regression phase was observed at 9 days and was more common in injuries more than 2 weeks old.

In contrast to our study, Siddiqui *et al*.[5] observed an earlier regression phase from 7 days up to more than 2 weeks. Dimaio[13] quotes that the regression phase will start by the 12th day.

This study found a statistically significant relationship between the age of abrasion and histopathology findings (X^2_{81}=552.92, P<0.001). A similar statistical significant relationship between the two was reported by Vinay J *et al*. (X^2_{24}=99.37, P<0.001).[4]

CONCLUSION

We found an ordered and sequential appearance of cells in healing abrasions. Congestion of vessels and haemorrhage were seen in abrasions that were <5 hours old. Edema to present up to 35.75 hours. Polymorph to appear earliest at 1.67 hours, and predominant polymorph infiltration up to 29.08 hours. On the third day (27.08–69.5 hours) after wounding, mononuclear cells were visible microscopically. On the fourth day (72.17–92.17 hours) fibroblast appears. From the fifth to seventh day, deposition of granulation tissue occurs. Collagen formation was seen in abrasions between 157 and 333.33 hours (7th to 14th days). Regression phase starts only after 14 days. The relationship between the age of abrasion and histopathology findings was found to be statistically significant (X^2_{81}=552.92, P<0.001).

LIMITATIONS

Because of random sampling, the majority of samples in our study were from cases brought within 6 hours of wounding.

RECOMMENDATIONS

Naked eye examination (subjective) of gross changes in abrasions, gives only a rough estimate regarding the age, hence, subjecting the samples to histological examination for corroboration is recommended. Histopathological correlation for dating of abrasions is particularly useful in sensitive cases that demand a more accurate determination of the age of the injury. Research with a larger sample size, to improve external validity, is recommended.

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