

Histopathological Study of Blunt Force Injuries in Relation to Time Since Death

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ABSTRACT

Introduction: Accident/injury can happen to anyone at anytime. It may lead to death. Nature of wound is to be ascertained and age of the wound is to be estimated and having more forensic pathology significance. The present study was done to determine the age of blunt injuries by histopathological techniques and to correlate histopathological changes in relation to time of injury.

Material and methods: The study conducted at Osmania General Hospital during 2012-2014. All fatal cases having well demarcated wounds with known and unknown time of injury were included among the cases subjected to medico legal autopsy. Standard format was used in collection of data. 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 and relevant details of the injuries like position, size and color were taken into consideration and the age of injury was assessed depending on appearance. Then injuries were grouped 7 different time intervals. Affected areas of wound including adjacent normal skin sent for histopathological studies.

Results: 215 study subjects were taken in the study through both inclusion and exclusion criteria. 106 cases were in the age group of 20-60 years. Survival time after injury in 34% of cases was less than four hours. 33% of cases came with lacerated wounds. 85% cases were admitted with road traffic accidents.

Conclusion: In 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 particularly when the age of the injury is in dispute.

Keywords: Wound, Histopathology, Autopsy, Blunt force.

defendable in the court. The present study was to determine the age of blunt injuries by histopathological techniques and to correlate histopathological changes in relation to time of injury. The purpose of the current study was to determine the age of blunt injuries by histopathological techniques and to correlate histopathological changes in relation to time of injury.

MATERIAL AND METHODS

The present study was conducted in the Department of Forensic Medicine, Osmania Medical College/Osmania General Hospital from October 2012 to March 2014, for a period of 18 months. All fatal cases having well demarcated wounds with known and unknown time of injury were included among the cases subjected to medico legal autopsy. A standard pro-forma was used to collect information regarding time of injury, time of death and associated co morbidities. 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 and relevant details of the injuries like position, size and color were taken into consideration and the age of injury was assessed depending on appearance. Then injuries were grouped into 7 different time intervals: Injuries of 0-4h, 4-12h, 12-24h, 24-72h, 4-6 days, 7-14days and more than two weeks old. Then the representative areas of injury with adjacent normal skin were sampled along with control sample from nearer to the tissue of injury, and subjected for histopathological examination (figure-1).

The samples were transferred into a cassette. This was immersed into multiple baths of progressively increasing concentrated ethanol to dehydrate the tissue. It was followed by toluene or xylene and finally extremely hot paraffin. During this 12-16 hour process, water in the tissues was replaced by paraffin. This paraffin turned soft moist tissues into a sample which formed a homogenous mixture with paraffin. Tissue processing was the term applied to this method. From the cassette the processed tissue was taken out and set into a mould.⁴ Extra paraffin was added through the process of immersion, to make a paraffin block which was secured to the container from outside.

Cutting of the tissues by the process of implanting, into

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very slim (2-7 micrometer) sections was done by using a microtome.⁵ The slices were thinner than the average cell and were layered on a glass slide for staining by hematoxylin and eosin (often abbreviated as HandE). Then the slide was viewed under microscope. Von Gieson's staining was done wherever necessary to confirm the presence of collagen tissue.

Inclusion criteria: Time of infliction of injury was known or not known were taken as cases and included in the study.

Exclusion criteria: Bodies in the state of decomposition were excluded from the study.

Specimen size

The present study 'Histopathological Study of Blunt Force Injuries in Relation to Time since death' was carried out in the Department of Forensic Medicine, Osmania Medical College/ Osmania General Hospital, Hyderabad from October 2012 to March 2014, for a period of 18 months. From the retrospective analysis of records of previous 18 months revealed that 233 case samples were fulfilling the inclusion criteria. Thus after considering a 10% loss, it was decided that the minimum number of case samples required for the study to be significant was 210 case samples. However, a total of 215 case samples (injuries) fulfilled the criteria and were taken up in the study. An equal number of controls for the injuries studied were included.

STATISTICAL ANALYSIS

Results of the study are based on the descriptive statistics like mean and percentages. Microsoft office 2007 was used to generate statistical data and tables.

RESULTS

The post mortem "Histopathological study of blunt injuries in relation to time since death" was carried out at the Department of Forensic Medicine, Osmania Medical College/Osmania General Hospital, Hyderabad from October 2012 to March 2014. A total of 128 cases (215 injuries) were taken up for the study.

In the study out of 128 cases, 111 cases (86.7%) were males since they are more into outdoor activities like driving vehicles, labour workers etc. and the remaining 17 cases (13.3%) constituted females. Those in the susceptible age group were 21- 40 years (61 cases) followed by age group of 41- 60 years (45 cases). The reason being that, they form the work group and hence more prone to injuries due to road traffic accidents, fall, assaults, etc.

Among 128 cases, 44 (34.4%) cases were 0-4 h old, 12 (9.4%) cases were of duration 4 -12 h, 11 (8.6%) cases were 12-24 h old, 21(16.4%) cases were 24-72 h old, 10 (7.8%) cases were 4-6 days old, 19 (14.8%) cases were of 7-14 days and the remaining 11(8.6%) cases were > 2weeks old (table-1).

Distribution of types of blunt injuries

A total of 215 case samples (injuries) were studied from amongst the 128 cases, comprising of abrasions 101(46.98%), contusions/ bruises 44 cases (20.47%) and lacerated wounds 70 (32.56%) (table-2).

Gross changes

101 abrasions were studied, amongst which 33 (32.7%) showed bright red, 22 (21.8%) reddish scab, 16 (15.8%) brownish scab, 3 (3.0%) dark brown scab, 10 (9.9%) black scab and in 13

(12.9%) injuries the scab was fallen off at margins. In 4 (4.0%) injuries the scab had fallen off completely.

Bright red colour was observed in 33 abrasions, 29 (87.9%) of these injuries were 0-4 h old. The remaining 4 injuries were of 4-12 h old, more so they were in 4-5 h and were located in the less vascular areas of the extremities. No co morbidities were associated in any of these cases. Bright redness was observed earliest at 10 min and latest upto 5 h.

Reddish scab was noted in 22 injuries, of which 8 (36.4%) injuries were 12-24 h old. The remaining 14 were distributed equally (31.8% each) in the duration 4-12 h and 24-72 h. Reddish scab was observed earliest at 6 h and latest upto 68 h.

Brownish scab was noted in 16 injuries, of which 10 (62.5%) injuries were of 24-72 h, the remaining 2 were 12-24 h old and 4 were 4-6 days old. The earliest brownish scab was noted at 18 h in 2 injuries, where both injuries were small size and the deceased were young who did not have co morbidities. The latest appearance of the brownish scab was at 132 hour (5.5 days).

The dark brown scab was noted in 3 injuries, of which 2 (66.7%) injuries were 4-6 days old and the remaining was 24-72 h old. The earliest dark brown scab was noted at 44 h, where the injury was situated over face (more vascular area). The other 2 injuries were at 97 h and 144 h, however their sizes (2x1 and 5x3cm)

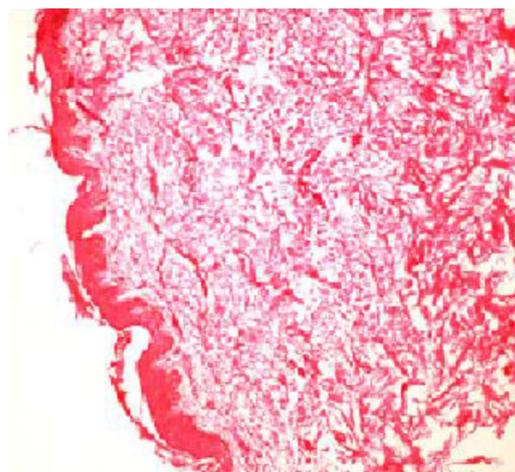


Figure-1: Low power microphotograph: Section shows thinned out epidermis and dermis and dermis shows increased fibro-collagenous tissue with congested blood vessels.

Microscopic changes	Earliest appearance	Routine appearance
Congestion/ hemorrhage	10 min	0-4 h
Oedema formation	15 min	0-4 h
Margination of polymorph cells	30 min	0-4 h
Early infiltration of neutrophil cells	6 h	4-12 h
Predominant neutrophilic infiltration	12h	12-24 h
Mononuclear cell infiltration	24 h	24-72 h
Fibroblast formation	71 h	71-78 h
Granulation tissue deposition	72 h	4-6 days
Collagen formation	96 h (4 days)	7-14 days
Regression phase	213 h (9 days)	>2 weeks

Table-1: Earliest and routine appearance of common histologically detected changes of abrasions.

Age of injury	Microscopic scoring											Total
	0	1	2	3	4	5	6	7	8	9	10	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
0-4 h	23	1	5	0	0	0	0	0	0	0	0	29
4-12 h	6	0	3	2	0	0	0	0	0	0	0	11
12-24 h	1	0	0	1	8	0	0	0	0	0	0	10
24-72 h	0	0	0	0	7	6	1	4	0	0	0	18
4-6 d	0	0	0	0	0	0	1	5	2	0	0	8
7-14 d	0	0	0	0	0	1	0	3	7	4	1	16
>2 weeks	0	0	0	0	0	0	0	1	1	6	1	9
Total	30	1	8	3	15	7	2	13	10	10	2	101

Score: 0- Haemorrhage and/or Congestion of vessels; 1- Oedema formation; 2- Margination of polymorph cells; 3- Early infiltration of neutrophil cells; 4- Predominant neutrophil infiltration with poorly differentiated mononuclear cells; 5- Predominant mononuclear cell infiltration; 6- Fibroblast formation; 7- Granulation tissue with rich leucocyte infiltration and much fibroblasts; 8- Collagen tissue deposition; 9- Cellular reaction subsides, fibroblast are more active with increased collagen formation (regression phase); 10- Organized pustule

Table-2: Distribution of abrasions by its microscopic changes

were small and deceased did not have any co morbidities.

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

Scab fallen at the margin was seen in 13 injuries, of which 8 (61.5%) injuries were of 7-14 days and remaining 4 were > 2 weeks old. The fall of scab was noted only after 6 days and was seen upto 15 days in healthy individual where injury was located in the lower extremity (knee). In remaining 3 cases there was significant delay of fall of scab observed upto 45 days. All 3 of them were suffering from septicemia.

In 4 injuries, the scabs had fallen off completely, of which 3 (75.0%) injuries were >2weeks old and in the remaining injury, the scab had fallen off at 11 days itself. In healthy individual it took upto 17 days for the scab to fall off completely. However in a comatose, who had comorbidities such as diabetes and septicemia, fall of scab (complete) was observed on 27th day.

DISCUSSION

Dating of an injury both in living and dead is of crucial medico-legal value in the field of crime investigation to fix the responsibility. Forensic experts are often asked to give their opinion on the age of wounds⁸ and the interpretation may have significant medico-legal consequences.¹⁰

In the present study, reddish scab was observed earliest at 6 h and latest upto 68 h. This was in agreement with the study conducted by Sharma A et al⁶ where he found redness from 10 min upto 7 hours. In contrary it was observed in another study that majority of cases on the first day were dark red instead of bright red.⁸ Reddish scab was observed earliest at 6 h and latest upto 68 h. Similar observation was made by Sharma A et al⁶ in their study the author also quotes that reddish scab forms by 8-24 h. The latest appearance of the brownish scab was at 132 h (5.5 days). Similar observation was made by Sharma A et al⁶ and author also quotes that the scab looks brownish by 2nd and 3rd day. This was in disagreement with the study conducted by Kumar L et al³ wherein they observed that on 3rd day, majority

of injuries were dark red in colour instead of brownish. Sharma A et al⁶ found hard brown scab 27 h onwards and the author also quotes that by 4th and 5th day the scab looks dark brown. However this was in disagreement with another study,⁴ where they observed dark red scab instead of dark brownish scab on 5th day.⁹

Wound dating was done by macroscopic/gross appearance, microscopic examination and by histochemistry techniques. In Indian scenario, usually, we adopt the "naked eye examination" method to date an injury. It is difficult to determine exactly the age of an injury on the basis of its appearance by the unaided visual inspection. Because of the change in the intensity of the local inflammatory reaction the results obtained are inaccurate.⁹ There is obvious incongruity existing in the standard textbooks, sufficient enough to pose difficulty for dating of mechanical injuries.³

In the evolution of medical field and in the administration of justice, histopathological examination of wounds provides a high degree of reliability. There are many literatures on this subject, as with time of death, it can be an important matter in forensic medical investigations to determine whether a wound found at autopsy was inflicted before death and, if inflicted ante mortem, how long before death it was sustained. Unfortunately, as with so many problems, biological variability introduces a wide margin of uncertainty. Numerous surveys and classifications exist in which histomorphological findings and criteria towards the determination of age. The changes will also vary according to the size of a wound, the type of wound, the tissue, age and health of the victim and whether there is infection.¹

To determine the age of injury, the exact knowledge of the timing of the repair processes is needed. Skin-wound healing is an orchestrated biological phenomenon consisting of three sequential phases, inflammation, proliferation, and maturation. Many physical, histological and chemical changes are involved in the process of wound repair, and this short and simplified overview of wound healing can be adopted to determine wound age.⁹ Fall of scab, in the present study (complete) was observed on 27th day. In a similar study by Sharma A et al⁶ found that, the black scab started separating 7 days onwards and it was blackish by the 6th day and it began to shed off from the margins. It took a few more days for the large scab to shed off.⁷ However this was in disagreement with the study,⁸ where they observed dark

brown scab instead of black scab on the 9th day.

It is essential to study the injuries microscopically because of the ambiguous and variable results of the unaided visual examination of the wounds. Comparing the gross appearance with histological changes of wounds is still important in spite of the complex methods like immunohistochemistry and histochemistry for dating of the injury.^{8,10}

Analysis of the literature shows an appreciable variation in the time periods associated with injury development and appearance and that there is variation in rates of wound healing in different sites of the same individual. 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.

The macroscopic and microscopic study of dating the injury was taken up in our department. This study was done to check the accuracy of injury dating and correlating with the age determined by colour changes. The microscopic examination of wound was correlated with the time of occurrence of injury as per the hospital/ FIR/ police records.

Limitations

In individuals with dark to light brown complexion the colour changes in the contusions were not appreciated clearly and more so this type of naked eye observation is subjective.

As only one observer was involved in the study, the degree of possible observer variation could not be assessed.

Scope for further study

Further studies on enzyme histochemistry, biochemical assays and fluorescence studies can be done and can be correlated with microscopic changes. This will help in removing subjectivity and bring more objectivity in the observation and interpretation. For colour identification of bruises spectrophotometry can be used in living subjects.

As gross changes of lacerations are not consistent with dating of injury, the nature of laceration can be considered for further study.

CONCLUSION

To conclude, though gross features of the injuries provides a rough estimate regarding their age, histological examination will confirm or gives a more accurate timing of their occurrence, thus aiding in administration of justice.

RECOMMENDATIONS

In cases with multiple injuries over different sites, the healing process may vary due to 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 artefacts in the form of sand, mud particles, glass pieces etc. are to be kept in mind.

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